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VOL. XXIV FOR 1953

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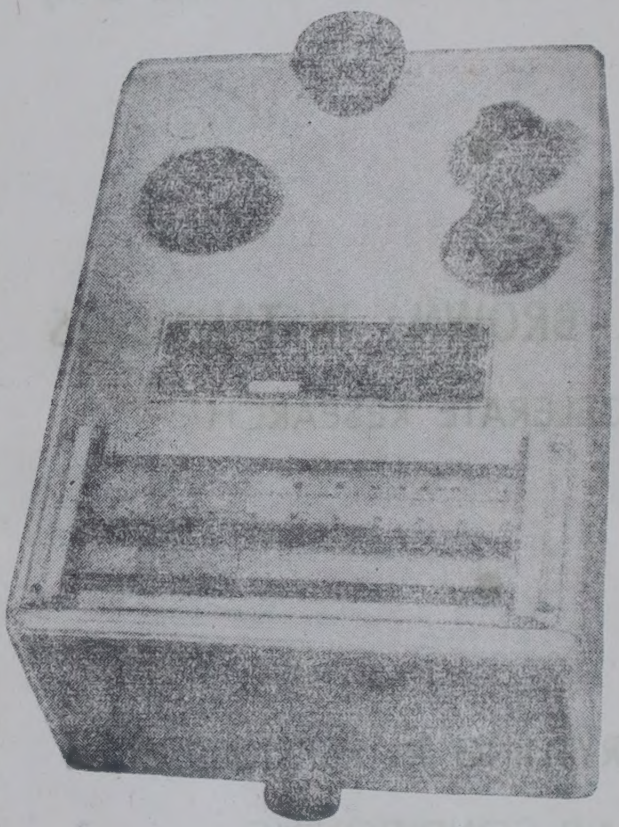

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* The Society is very grateful to the Council of the National Institute of Sciences of India for a generous grant for meeting a part of the cost of printing this Review.

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RESPONSIBLE FOR OPINIONS EXPRESSED
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CHROMATOGRAPHY

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During the period since the previous review, a large number of publications in the field of chromatography and in its application to biochemical and other problems have appeared. A symposium on 'Chromatography' was held in Bangalore in April 1953 and the abstracts of papers presented at the symposium have been published by the Society of Biological Chemists, India. [1]

Paper Chromatography

Theory and Technique: In a detailed paper on the partition mechanism of paper chromatography Burma [2] has studied in particular the role of cellulose. He considers that adsorption plays a significant role at least in some cases. The first step in chromatographic separation is assumed to be the formation of a three- instead of a two-phase system consisting of: (*a*) water chemically bound to cellulose; (*b*) free adsorbed water and (*c*) the bulk of non-aqueous liquid water. Two types of distribution of the solute molecules come into play: (i) distribution between the second and third phases, which is a case of pure partition and (ii) distribution between the first and the second phases, which may be looked upon as an adsorption process. Although partition is the primary process responsible for the separation of constituents of a mixture on a chromatogram, the role of "adsorption" though of secondary importance in most cases, cannot be neglected.

An elegant experiment of Chakraborty and Burma [3] has confirmed the assumption of Rao and Giri [4] that orthogonal distribution of the solvent is responsible for the higher Rf values observed in circular paper chromatography.

Rao and Dickey [5] have modified the earlier horizontal migration method for simultaneous identification of substances, by placing a mixture of known and unknown ones near the centre, at the

two corners where the tail joins the rest of the paper. The advantages of this modification over the method of Giri and Rao [6] are not obvious, especially when more than two samples are to be analysed on the same chromatogram.

Mohan Rao [7] suggested a method for increasing the effective distance travelled by the solvent front and it consists in shifting the position of the wick from the centre to a position near the periphery of a paper disk (18.5 cm.). A maximum of only about three samples could be analysed. The amino acids cystine, glycine and glutamic acid separate well even on a 25 cm. paper disk (running time 4-5 hours) by the central wick method.

Siddappa and Bhatia [8] have suggested a simple device for keeping cylinders of paper inside glass cylinders. A circular filter paper is folded into a cone and then placed with the tip of the cone inside the paper cylinder and the rim flush with the top of the glass cylinder. The cylinder is closed by a ground glass cover.

Krishna Pillai [9] has extended the multiple development technique of Jeanes *et al* [10] and has found good agreement between the observed and calculated values for the number of runs necessary for the maximum distance of separation of as many as five sugars.

Venkatesh *et al* [11] have used suitably washed sewing thread for developing an ultra-micro method for the fractionation, characterisation and estimation of antibiotics, vitamins and other active principles.

Using a new principle, a method for the separation of organic compounds has been developed by Vasisth and Muthana. [12] The mixture of substances to be resolved was dissolved in a volatile solvent and deposited on paper. The paper being enclosed in a bell-jar was then developed with a pure solvent or a mixture of solvents. Several mixtures of organic compounds have been separated by scraping off the components deposited at different levels on the paper.

Giri *et al* [13] have extended the study of the quantitative estimation of amino acids by circular paper chromatography. Details of the variables involved in the ninhydrin reaction have been described. The effect of metallic ions, concentration of ninhydrin reagent, solvents for ninhydrin, temperature and time of incubation

and the area of the bands on the intensity of the extracted colour, have been investigated. Traces of Cd^{++} and Cu^{++} markedly affect the colour of the ninhydrin pigment and this property has been utilised for establishing the optimum conditions for the quantitative procedure. This method has been employed for the estimation of some of the amino acids in edestin. One of the chief drawbacks of this procedure is the difficulty of resolving some of the overlapping amino acids by circular paper chromatography. However, Giri and Rao [14,15] have attempted to separate them by using different solvent systems. For qualitative work, the strip containing the overlapping amino acids, from a chromatogram using butanol solvent, was used as a wick in a second chromatogram. This was developed with pyridine-water (80 : 20) as solvent. Glutamic acid, threonine, serine, aspartic acid and glycine have been separated. The method has been rendered quantitative by chromatographic elution of the amino acids from the strip to another paper disk and estimation after development with the second solvent.

Rao and Giri [16] have described a method for the separation, identification and estimation of urea in human urine. The bands relating to urea, marked by the aid of guide strips sprayed with phenol-hypochlorite reagent, are cut out, eluted with water and estimated colorimetrically using α-isonitrosopropiophenone.

Giri and Nigam [17] have developed a method for the separation of simple saccharides and oligosaccharides using n-butanol-acetone-water (20 : 70 : 10) and n-butanol-pyridine-water as solvent mixtures. Multiple development greatly improved the resolution of sugars. A quantitative method for reducing sugars using triphenyl tetrazolium chloride (T. T. C.) is also given by the authors. [18] The method consists of development of the chromatogram and of applying the T. T. C. reagent with a swab of cotton. The paper is then kept in a humid atmosphere under prescribed conditions. The red coloured bands were extracted with 95% alcohol and estimated using a Klett-Summerson Photo-electric colorimeter.

Applications

Conventional methods: Among the water-soluble carbo-

hydrates in the stem of *Agave vera cruz* Srinivasan and Bhatia [19] have detected fructose, sucrose, four unidentified substances possibly oligosaccharides and two polyfructosans. Bhatia *et al* [20] have shown that the underwood contains at least seven water-soluble carbohydrates. Polyfructosan was the major component as adjudged by visual comparison. Srinivasan and Bhatia [21] in a subsequent paper have determined the make up of carbohydrates of the stem and pole of *Agave*.

Srinivasan *et al* [22] studied the carbohydrates of garlic and onion and drew attention to the similarity in pattern in such widely differing sources as the tubers of Jerusalem artichoke (*compositae*), the stem of *Agave* or the bulbs of *Allium* and to its significance in the understanding of the synthesis of polyfructosans which form the reserve plant food.

Govindarajan [23] has applied papyrography in the study of raffinose-melibiose fermentation by yeasts. A direct proof for the presence or absence of melibiose in beers from yeast fermentation of raffinose broths is obtained and is useful in screening a large number of cultures for purposes of classification.

Das *et al* [24] have identified xylose, glucose and arabinose in the formic acid hydrolysates of α-cellulose. In the case of hollocellulose a new prominent spot in between the spots of uronic acid and glucose has been detected. Majumdar and Sarkar, [25] however, could detect neither xylose nor arabinose in the above case.

Srivastava [26] has examined the sugars in some common Indian fruit juices. Fructose and maltose occur in orange, pomegranate, mango, lichi, black plum, melon, *falsa* and jack fruit. An oligosaccharide has been isolated from *falsa*-juice.

Venkatachalamurthy and Sreenivasaya [27] have described a papyrographic method for the detection, characterisation and estimation of Ketobodies in the haemolymph of the silk worm (*Bombyx mori*). Pyruvic acid and three more unidentified Ketobodies are present. α-Ketoglutaric acid, however, could not be detected.

Roberts and Wood [28] have extended their work on the separation of polyphenols of the tea leaf. It is interesting to note

that water was used as the mobile solvent and the separation of the optical antipodes of catechin, epicatechin and gallo-catechin and epigallocatechin appears to be achieved. Aglycones of flavanols have zero Rf in water and may then be distinguished from their glycosides. They conclude that the use of water as a solvent is of some theoretical interest as the separations achieved are apparently due to adsorption rather than to partition effects.

Roberts, Ayengar and Posner [29] have employed one-dimensional chromatography for the estimation of amino acids in reactions involving transamination of γ-amino butyric acid and β alanine, in micro-organisms.

Das and Biswas [30] have studied the amino acid content in wheat gluten and mango seed kernel.

Employing paper chromatography and ionophoresis, Ghosh and Burma [31] have identified adenine, guanine, cytosine and uracil as constituents of yeast nucleic acid.

Phatak and Patwardhan [32] have identified the iso-oletic acids in cow and buffalo milk fat.

Radhakrishna Murthy and Sarma [33] successfully separated and identified most of the B-group vitamins by the ascending technique using n-butanol-methanol-benzene-water (2 : 1 : 1 : 1) as solvent. They found that dilute acidified $KMnO_4$ solution gave a yellow spot of biotin sulphone even when 4 μg. of biotin were present.

Agarwala *et al* [34] have studied the enzymatic hydrolysis of glutathione by bacteria (*Vibrio cholerae*) using the ascending method. They found that the cysteine moiety was taken up or metabolised by the system.

Reddy and Kodicek [35] have applied chromatographic procedures for the separation of tertiary and quaternary nicotinyl compounds in urines and the concentrations of the tertiary nicotinyl compounds were evaluated by visual comparison of the coloured spot. The excretion of metabolites by man and the rat after administration of nicotinic acid, nicotinamide and tryptophan has been studied.

As a preliminary note in their experimental study of chromatograms of the urine of rats treated with various liver poisons, Wahi

and Nigam [36] have prepared a list of Rf values of thirty-four ninhydrin positive substances using phenol-water as solvent.

The influence of ACTH administration in the human on the urinary excretion of nitrogenous substances was investigated by Ronzoni, Roberts, Frankel and Ramasarma.[37] The hormone caused a remarkable increase in the excretion of amino acids, ammonia, urea, uric acid and some undetermined nitrogenous constituents. Two-dimensional chromatograms revealed some of the detailed changes in the excretion of both free and bound amino acids. Microbiological assays of some amino acids, gave results which were in concordance with the chromatographic findings. All the urinary nitrogenous constituents returned to normal levels when the treatment was discontinued.

Circular Paper Chromatography

The usefulness of the circular technique is being increasingly recognised and the number of applications in diverse fields bear testimony to its superiority in certain respects, over the conventional methods.

Giri *et al* [38] found that several amino acid solutions when exposed to sun-light in presence of a photosensitizer (TiO_2) gave rise to a number of ninhydrin positive substances. Many of them were identified as amino acids. A provisional scheme for the photolytic decomposition of glutamic acid is given and they assume in this scheme the oxidation of the terminal CH_3-group. In a recent note the authors [39] have observed that the oxidation of methionine in presence of H_2O_2 is somewhat similar to its photolytic decomposition.

Giri and Kalyankar [40] observed that non-enzymatic transamination took place on paper at elevated temperatures. Glutamic acid and alanine were formed from a number of amino acids in presence of Na-α-ketoglutarate and -Pyruvate respectively. Giri *et al* [41] contend that this transamination reaction is substantially different from the well-known transamination of Herbst. It has been noticed that it is essentially a dry reaction although just traces of moisture may be necessary to initiate the reaction.

The method of Giri and Nigam [18] has been applied to the studies on the mechanism of amylolysis by Giri *et al* [42] The chromatograms of β-amylolysis show only one prominent maltose band while those of salivary α-amylolysis show in addition other bands of higher saccharides. The Taka-diastase hydrolysis showed that the short dextrins formed, at the initial stages disappear giving rise to higher saccharides, indicating resynthesis.

Narasimha Rao and Venkataraman [43] in their studies on the nitrogen metabolism of *Penicillium chrysogenum* Q—176, observed that at the peak period of formation of penicillin there was rapid disappearance of virtually all amino acids in the broth filtrate while there did not appear to be correspondingly marked variation in the mycelium. Their finding lends support to the view that the formation of penicillin is intimately connected with the utilisation of amino acids which have accumulated during the early stages of growth.

Pillai *et al* [44] from a study of the amino acids in sewage and activated sludge conclude that raw sewage contains almost all the essential amino acids and during purification by the activated sludge process the amino acids are concentrated in the sludge, the purified effluent being practically free from amino acids.

Krishnaswamy and Giri [45] observed the presence of γ-amino-butyric acid in the acid hydrolysate of a red yeast *Rhodotorula glutinis* (Fres). They have traced the pathway for such a metabolite and the enzyme system involved and have suggested the possibility of the formation of γ-aminobutyric acid in yeast by enzymatic decarboxylation of glutamic acid.

Giri *et al* [46] have investigated the effect of autoclaving proteins with glucose. They found that when casein was autoclaved with glucose, arginine was completely destroyed and this destruction in the case of albumin, gelatin and fibrin was less. The destruction of lysine and histidine on a combined basis was of the order of about 50%. The significance of these observations has been discussed.

Kulkarni [47] has studied the essential amino acids of silver bar fish (*Chirocentrus dorab* and *meretrix*).

Airan and co-workers [48-54] have extensively used circular

paper chromatography in the study of amino acids of fish protein and other materials (Scylla, lobster, human finger nail, pulp of the fruit *Pithecolobium Dulce* (Benth). Although the complete amino acid make up is not worked out and the significance of only the amino acid analyses in such diverse biological materials not discussed, the general applicability of the circular technique is suggested.

Kamala Sohonie and Hatwalne [55] have detected glutathione in *neera* and by estimating glutathione with p-chloromercury benzoic acid and "SH" compounds by iodometric method, they have found that the 'SH' compounds in *neera* exist entirely as glutathione.

Sundaram and Sarma[56] and Sundaram *et al* [57] in their studies on the tryptophan metabolism in rice moth larvae (*Corcyra cephalonica* St.) have shown that kynurenine and 3-hydroxykynurenine constitute the yellow pigment excreted by the larvae on a tryptophan-rich pyridoxine-deficient diet. The absence of xanthurenic acid in the excretory pigment has also been demonstrated.

Singh and Chandy [58] have employed the circular technique for the amino acid analysis of the cerebrospinal fluid from epileptic patients. Comparing the normal brain chromatogram with that of the epileptogenic foci, they found only quantitative differences in their amino acid pattern.

Gauranga Ray *et al* [59] have identified the amino acids in purified prothrombin. It is not clear how the overlapping amino acids were identified by running only mixed chromatograms.

In a polemical note, Giri [60] has discussed the suitability of circular paper chromatography for the analysis of any biological material.

Airan *et al* [61] have used a 0.1% solution of mercurochrome in alcohol for the identification of organic acids. Employing this method they have examined the organic acids in *Lycopersicum esculentum*. Airan and Barnabas [62,63] have effectively separated maleic and malonic acids by the two-dimensional circular chromatography using pentanol 5 M-formic acid (40 : 50) as solvent.

The effect of exogenous biotin on the utilization of glutamic acid by two strains of yeasts, BY2 and *Rhodotorula glutinis* has

been studied by Krishnaswamy and Giri.[64] BY2 for which biotin was a critical growth factor assimilates glutamic acid in the presence of biotin only, whereas in the other case biotin enhanced the rate of assimilation. The greater stimulatory effect of biotin on the metabolic function of BY2 is indicated.

Ganguli[65] separated some phosphoric esters using n-butanol acetic acid-water (4 : 1 : 5) as solvent. The use of 0.1% solution of ascorbic acid in place of exposure to vapours of H_2S gave improved results. In a recent note[66] he used this method for the identification of phosphate esters present in rat liver tissue.

Giri *et al*[67] described a method for the separation and identification of the acid hydrolytic products of purines and nucleic acids The formation of a compound, tentatively identified as 4 (5) amino-5 (4) imidazole carboxamidine, from adenine and nucleic acids even under mild conditions of hydrolysis is noteworthy.

Giri[68] has described a simple paper chromatographic method for the estimation of vitamins B_1 and B_2. B_1 was converted into thiochrome by means of cyanogen bromide and ammonia and was separated from B_2 using Partridge's solvent. The estimation was carried out by cutting out the fluorescing bands and measuring the fluorescence after elution, in a Klett-fluorometer

Puri and Seshadri[69] have applied the horizontal migration method to the study of typical flavanones and their naturally occurring glycosides. With this method they examined the crude products of hydrolysis and showed that hesperidin and naringin yield only the flavanones (hesperetin and naringenin respectively) and that butyrin yielded a mixture of butein (chalkone) and butin (flavanone). They detected a new chalkone glycoside in the extracts of *B. frundosa* flower. Ahluwalia *et al*[70] have identified the isoflavones genistein and daidzein in soyabeans using alcohol-water (1 : 3) as solvent.

Miscellaneous: Rahman and Minhaj[71] have attempted to purify insulin by chromatography on a column of activated alumina and by elution from it by picric acid and acidic alcohol. They found that insulin picrate which was supposedly homogeneous gave rise to three bands.

By paper chromatography, Viswanathan [72] has achieved the separation of alkali chlorides using rectified spirit and ammonia as the developer.

Burma [73] has successfully separated by paper ionophoresis, the purine and pyrimidine bases adenine, guanine, cytosine, thymine and uracil. Thymine and uracil which do not separate under acid conditions, have been separated using alkali solution as the conducting medium.

Rao [74] has devised a simple inexpensive apparatus for micro electrophoresis. Using Whatman No. 3 paper he has separated the serum proteins.

Lakshmanan and Liebermann [75] have described a method for gradient elution chromatography. A Hershberg dropping funnel connected to a mixing chamber having a magnetic stirrer is a suitable apparatus. A curve showing the resolution of a mixture of five 17-ketosteroids in a concave elution chromatogram is given.

A column chromatographic method of estimating phosphates in small quantities has been employed by Rangarajan *et al* [76] for the estimation of P_2O_5 in bone ash. The chromatogram is developed with nitro-ammonium molybdate and the yellow phosphate band is compared with a similar band produced by a standard solution.

Radhakrishna [77] has separated ^{141}La from ^{90}Y, Sc from La and La from Y by adsorption on Amberlite IR-100H ion-exchange resin and subsequent elution with 5% citric acid at two different pH values, 2.8 and 3.2.

Electrochromatography has been employed by Burma [78] for the study of a number of amino acids at various pH values with a mixture of phenol and a buffer solution as the developing solvent. By a choice of suitable pH and application of electric field in the proper direction amino acids having close Rf values (except isomers) can be separated from one another.

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FOOD TECHNOLOGY

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Food Technology, a branch of science which deals with various chemical and technological aspects of foods, was not given adequate attention in this country prior to World War II. During the war years, the chemists and technologists were called upon not only to conserve the available resources of foods but also to evolve and develop 'ersatz' products for those which were either imported or otherwise scarcely available. With the establishment of Central Food Technological Research Institute at Mysore, adequate facilities have now been provided for co-ordinated research on various integrated aspects of foods and during the past 4 or 5 years considerable work has been done in food technology and allied fields.

In the present review an attempt has been made to consolidate the available literature published during the year. For convenience, the subject has been classified under seven broad divisions, namely,

(1) Cereal Technology, (2) Fruits and Vegetable Preservation, (3) Coffee, Tea, Confectionery, (4) Storage Preservation of Foods, (5) Milk Substitutes, (6) Fish Technology, and (7) Miscellaneous.

1. Cereal Technology

Subrahmanyan *et al* [1,2] have developed a protein-rich invalid food (Tonic Food) form a suitable blend of maize starch, casein, defatted groundnut flour, food yeast, vitamins and minerals. The product has been prepared in the forms of rice-shaped grains, semolina-like product and fine flour. Subba Rao, Bains, Bhatia and Subrahmanyan [3] have investigated the possibility of developing a simple technique by which wheat, jowar and maize whole grains

could be rendered palatable and amenable to easy cooking. They observed that the best way of utilizing these grains is to admix their flour to tapioca flour to a level of 30% and to produce the product, "Synthetic Grains," from the blend. The blended grain possesses a distinct advantage over natural whole grain in that it cooks in 8-10 minutes. Subrahmanyan and his associates [4] have standardized the technique for imparting rice-shape to the 'Synthetic grains.' Pilot plant trials have been successfully concluded. The 'Synthetic rice,' now developed, can stand, unlike other paste goods, washing in cold water prior to cooking. Rama Rao, Murthy and Swaminathan [5] have worked out a method for preparing semolina-like product from soft wheat. The wheat is soaked in cold water for half an hour and parboiled by steaming at atmospheric pressure for 30 minutes prior to drying at 45-50°C in a blast of air. Sahasrabudhe and Bhatia [6] observed that the shelf-life of biscuits is appreciably increased when the melting point of the shortening is raised from 37°C to 42°C. Various aspects of the modern practice of enrichment of wheat flour have been reviewed by Sur and Iyengar.[7] Bains and Bhatia [9] have discussed several aspects of biscuit making with special reference to choice of raw materials, manufacturing technique and problems of adequate packaging. Some of the technical difficulties encountered in canning rice have been discussed by Bose.[8]

2. Fruits and Vegetable Preservation

In their investigation on orange juice Pruthi and Lal [10] studied the effect of different methods of processing, storage temperatures, sweetening, etc., on colour retention in 'Nagpuri' orange juice during storage. Sweetening of the juice with cane sugar at 5% level tends to aid darkening and there was greater deterioration in colour in benzoated samples than in sulphited ones. In his studies on grape-fruit juice also Pruthi [11] has observed that sweetening tends to accelerate darkening during storage. Tandon, Pruthi and Lal [12] have studied five varieties of potatoes namely, O. N. 208, Kerr's Pink, Mysore Ricket, Phulwa and Darjeeling Red for their suitability for canning and find that O. N. 208

belongs to 'Soggy' or 'Waxy' group while Kerr's Pink is improved upon by calcium chloride treatment. The other three varieties were not found suitable for processing. Jain, Bhatia and Lal [13] have standardized a method for the canning of vegetables such as potatoes, tomatoes, cauliflour and peas in combination with one another in 'curried' style. Moorjani [14] reported that fresh lotus rhizome, which is used as a vegetable and a pickling material contains 11% carbohydates and 2.7% protein. The seeds contain 18% protein and 50% starch on dry weight basis. Based on their pilot plant studies, Lal, Tandon and Pruthi [15] have estimated the cost of production of 'Sathgudi' orange squash at Rs. 1-10-8 per bottle. Siddappa [16] reports that 'Amte kayi' or Indian Hog Plum (*Spondias pinnata*, Kurz.) used for pickling contains only 6-10 mg.% of true ascorbic acid. Jain, Das and Lal [17] have worked out a method for the drying of 'Arvi' (*Colocasia*) and for the preparation of its flour. The Arvi flour when admixed with wheat flour in the ratio 1 : 3 can be used for making "chapatis". A method for the preparation and preservation of "Kanji" (fermented carrot juice) has been worked out by Anand.[18] When preserved with 0.03% sodium benzoate and 0.01% SO_2, the product can be stored for 6 months. Siddappa and Das [19] have shown that high percentage of alcohol insoluble solids as well as starch in peas are responsibie for gelation in canned green peas. In the case of over-mature peas, gelation can be minimized by decreasing the proportion of peas to brine. Preservatiou of orange juice with a view to eliminating the deterioration in flavour has been studied by Jain, Das and Lal.[20] The removal of scum which is formed on boiling the juice results in better retentiou of flavour during storage. Jain and Das [21] have reported that the maximum flavour rating of canned guavas is obtained by using a syrup of 35° Brix. The losses in vitamin C of the fruit due to the canning process and after storage at room temperature (25-30°C) for 6, 10 and 24 months were 19.4, 9.97, 18.3 and 39.55% respectively. Date and Bhatia [22] have developed a formula for the preparation of dehydrated vegetable soup. The soup powder can be completely reconstituted with boiling water in 2-3 minutes.

3. Coffee, tea, Confectionery

Kantharaj Urs, Natarajan and Bhatia [23] have reported that copper and zinc contents of coffee powder and 10% w/v brew range from 10-30 p.p.m. and 2-7 p.p.m. respectively in the powder and from 8-16 μg/100 cc. and 8-25 μg/100 cc. respectively in the brew. Bhatia and Natarajan [24] have reported the results of investigations on various aspects of coffee technology being conducted on behalf of Indian Coffee Board. The effect of admixing date seed powder with coffee powder on extractives, ash, caffeine, and chloroform extract has been studied. Ratio of *extractives to caffeine* and *extractives to ash* are useful for detecting adulteration. Extraction efficiency of different types of coffee filters has been determined. Extraction of a higher percentage of solubles has been achieved by adopting "wetting technique" which consists of wetting the powder with water to allow for swelling of the particles before passing hot water for extraction. In 'steeping' method, there is no significant difference in extractives among the ratio of 1 : 10 of powder to water and above; the differences are however significant below one to ten ratio. Natarajan and Bhatia [25] have discussed and reviewed the knowledge available on the vacuum packing of coffee. Caffeine content of certain samples of Indian Coffee has been found by Venkatachalam and Sundaran [26] to be as high as 2.2-2.6%. Mitra and Roy [27] have indicated the possibility of detecting chicory in coffee by colour reaction with Seliwanoff reagent. Chicory contains inulin hydrolysis of which gives levulose, while pure coffee does not contain levulose. Ramakrishnan, Balasundaran, Rajagopalan and Pillai [28] have prepared ion exchange material from coffee husk by suitable treatment with sulphuric acid, sodium chloride and alum. The material is efficient in removing calcium and fluorine from water.

Mitra and Roy [29] report that the estimation of crude fibre is useful in detecting woody stalks in tea and dust tea. Peter de Jong [30] has reported results of an investigation on the suitability of 'Alkathene' and 'Pliofilm' as lining materials for plywood tea chests. Tea packed in Alkathene and Pliofilm lined chests was reported by tasters as being 'dull,' 'flat' and 'soft.'

Desikachar, Bhatia and Raghunatha Rao[31] have studied the conversion of tapioca starch into syrup and observe that 0.2-0.25% concentration of acid gives low conversion syrups and that a concentration of 0.3-0.35% is suitable for producing high conversion syrups needed for table use. Date and Bhatia[32] have studied the shelf-life of an Indian sweet 'Besan Wadi' prepared from Bengal gram flour. Butylated hydroxy anisole (BHA) has been found to be effective in prolonging the shelf-life of the product. Some of the important developments in confectionery technology have been reviewed by Iyengar.[33]

4. Storage Preservation of Foods

Singh and Mathur[34] report that a temperature of 32-35°F and relative humidity (R. H.) of 80-90% are optimum conditions for cold storage of tapioca tubers (*Manihot utilissima*), the approximate storage-life being $6\frac{1}{2}$ months. The same authors[35] have reported that cashew apples can be stored for a maximum period of 5 weeks at a temperature of 32-35°F and R.H. of 85-90%. Anand and Johar[36] have observed that different varieties of raw mangoes intended for the preparation of chutneys can be kept for a maximum period of 40 days at 40-43°F and R. H. of 85-90%. According to Singh, Kapur and Mathur,[37] the optimum conditions for the cold storage of 'Totapuri' mangoes are a temperature of 42-45°F and R. H. of 85-90%, the storage life being 7 weeks. The mangoes thus stored can be ripened at a temperature 67-85°F. Mathur, Singh and Kapur[38] have recommended a temperature of 32-35°F and a relative humidity of 85-90% for the storage of shell eggs. The eggs obtained from White Leghorn and Barred Plymouth Rock breeds can be stored for 9 months under the above conditions. It has been reported by Mathur, Singh and Kapur[39] that seedling mangoes should be stored at 42-45°F with a R.H. of 85-99%. The mangoes intended for table use can be ripened satisfactorily in about 2 weeks at 62-65°F. In their studies on the cold storage of two varieties of potato namely, Up-to-Date and Hybrid-9, Mathur, Kapur and Singh[40] observed no sprouting at the end of 24 weeks in potatoes stored at 32-35°F and 35-38°F. Considerable sprouting, however, occurred at 42-45°F

and 47-50°F. The approximate storage life of potatoes under the optimum condition namely a temperature of 35-38°F and a R.H. of 85-90% was found to be 9½ months. According to Mathur, Singh and Kapur,[41] Raspuri (Peter, Naik, 1949) variety of mangoes can be stored for a maximum period of 42 days at 42-45°F in a green condition. Badami (Alphonso, Naik, 1949) can be stored only for 28 days at a temperature of 47-50°F. A temperature of 52-55°F and a R.H. of 85-90% have been recommended by Singh and Mathur [42] as the optimum conditions for the cold storage of Cavendish variety of bananas, the storage life being 22 days. Kapur, Mathur and Singh [43] have reported that a temperature of 59-87°F is suitable for the storage of onion sets.

Pingale and Muthu [44] have studied the control of rat damage in stored foodstuffs by using warfarin and tomorin (coumarine derivatives). They reported that neither of these two chemicals gave a complete solution to the rat problem but controlled the rat damage satisfactorily. Pingale [45] has observed that the viability of wheat and green gram (*Phaseolus mungo*) is affected by weevils and bruchids respectively; the weight of the grain was reduced by 36-38% in 6 months.

5. Milk Substitutes

Moorjani and Bhatia [46] found that during the steps involved in the manufacture of groundnut milk (Subramanyan, Moorjani, Bhatia; Indian Patent No. 47902), thiamine is lost to the extent of 16.78 per cent while a lower loss occurs in riboflavin and nicotinic acid contents. During the process of curdling (lactic fermentation) significant increase in the riboflavin content was noticed with some decrease in the nicotinic acid and no significant change in thiamine content. Nandi, Rajagopalan and De [47,48] have developed a method for the preparation of vegetable milk from a mixture of 56 parts of soyabean, 24 parts of groundnut and 20 parts of ragi malt. The milk compares favourably with cow's milk with respect to the over-all nutritive value and the availablity of calcium and phosphorus.

6. Fish Technology

The storage life of semi-dried prawns has been studied by Venkataraman, Sreenivasan and Vasavan.[49] Hard dried prawn (moisture content = 21.72 %) keeps well and no fungal growth was noticed even after 20 weeks when kept in sterilized 2 oz. glass stoppered bottles. Semi-dried prawns show extensive fungal growth and fading in colour by the end of 8 weeks. According to Venkataraman and Chari,[50] the edible portion of the Indian Chank (*Xancus pyrum*) which forms 3% of the total weight of the shell, contains about 24.84% proteins at a moisture content of 67.5%. It is also a rich source of calcium, iron and copper. Estimation of trimethylamine in marine fishes and fishery products is being adopted widely as an index of spoilage. Trimethylamine oxide is a precursor of the amine. Venkatraman and Chari[51] have found the presence of the oxide in 18 marine fishes of the west coast of Madras. Venkataraman and Sreenivasan[52] have observed that spoilage in mackerels preserved in oil is caused by the bacteria belonging to the genus Clostridium.

7. Miscellaneous

The carbohydrate make-up of the underwood of Agava vera cruz Mill has been studied by Bhatia, Satyanarayana and Srinivasan,[53] and Srinivasan and Bhatia.[54] The major portion of the carbohydrates of Agave consist of polyfructosans. Natarajan and Bhatia[55] and Subrahmanyan *et al*[56] have studied the processing of Agave and its utilization as an article of food. Cut pieces of Agave tuber can be made palatable by cooking in spiced tamarind water and considerable amounts of reducing sugars are released from the polyfructosans during cooking. The use of Agave as a vegetable has been indicated and it has been shown that the supplementary value of Agave to rice diet is due mainly to its calcium content. The qualitative make-up of water soluble carbohydrates in the bulbs of garlic and onions was found to be similar to that for Agave vera cruz by Srinivasan, Bhatia and Satyanaryana.[57]

Siddappa and Bhatia[58] have observed that it is essential that the pH of the reaction mixture should be adjusted to at least 4.0

for the estimation of ascorbic acid by xylene extraction method in preserved fruits and vegetables. Pruthi, Tandon and Siddappa [59] have reported that steaming for 60 minutes, with one change of water (at the end of 30 minutes) completely removes HCN from tender bamboo shoots. Siddappa and Raja Rao [60] reported the tin content of canned mangoes, grape-fruit, pummelo and pears in plain cans after 12-15 months of storage at 80-105°F. The tin content was found to be well within limits in all the fruits.

Chandrasekhara and Swaminathan [61] have reported that ragi malt when mashed with gelatinized cereal flours gives a higher yield of malt extract than when mashed by itself. The extract obtained from ragi malt and wheat flour possesses an agreeable flavour and is suitable for the preparation of malted milk powder.

Improvements in the manufacture of fruit juice powder by Siddappa and Lal,[62] process for improving the storage life of cashew kernels by Prasad, Kapur and Mathur,[63] a process for the preparation of concentrated foods from groundnut kernels by Krishna and Satyan,[64] improvements in the manufacture of fruit pulps in the form of sheets, slabs, etc. by Siddappa and Bhatia,[65] a solvent extractor by Raghunatha Rao [66] and a process for the deodorization and recovery of volatile principles from food materials by Krishna and Lakshminarayana,[67] are some of the developments which have been covered with patents.

A perusal of the above review will impress the reader with the keen iterest now being shown by chemists and technologists in the field of Food Technology. However, it may be pointed out that there is a considerable scope for more and more work particularly in fields of Fish, Cereal and Confectionery Technology.

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DAIRY SCIENCE

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Interest in the field of dairy science has been maintained during the year under review. The Food and Agricultural Organisation has brought out a valuable publication [1] on the Zebu cattle of India and Pakistan. They have assembled the already available information but what makes it of particular value is the information furnished on the environmental factors under which each breed thrives. A quarterly journal [2] devoted to the welfare of cattle in this country has been started by the Central Council of Gosamvardhan. The functions of Gosadan have been described by Harbans Singh.[3] The Indian Council of Agricultural Research has issued another useful publication dealing with animal nutrition research including dairy science in India up to 1948.[4] A review of dairy research in South Asia for the years 1946 to 1952 has been presented by Sen and Laxminarayana,[5] while a review of the progress of dairy chemistry in India has been made by Sen and Anantakrishnan.[6] Yegnanarayana Aiyer [7] has given an excellent account of dairying in ancient India. Quite a number of workers [8,9,10] stressed the importance of cow keeping and suggested methods for the improvement of milk supply. Paul [11] suggested that toning of milk is the most practical and quickest solution for the acute shortage of milk in India. Bhargava [12] has given an account of the present position of milk co-operatives in Uttar Pradesh, and dairy development in India during recent years has been summarised by Datar Singh.[13] The present position of the utilisation of carcasses of animals and need for its improvement are described by Valunjkar.[14] Warner and Agarwala [15] analysed the data regarding the training in animal husbandry and dairying imparted at the Allahabad Agricultural Institute.

1. DAIRY HUSBANDRY

Artificial Insemination

Sampathkumaran [16] discussed various factors pertaining to the

effective use of artificial insemination. The scope of and the results achieved in artificial insemination of cattle in the Punjab have been presented by Kohli and co-workers.[17] Krishna Rao [18] tried aqueous solutions of various strengths of a large number of dyes to determine their suitability or otherwise for staining bovine sperms. Some work on the differential staining of live and dead sperm and on the use of Leishman's, Wright's and Giemsa's stains for staining semen smears has been done by the same author.[19,20] Srivastava and co-workers [21] have shown that the *in vitro* life of the buffalo sperm could be prolonged 2 to 3 times by using only the active principle in egg yolk instead of the egg yolk itself in the dilutor. Results of 2,935 insemmnations made from 601 ejaculates collected from 13 *Bos indicus* and 8 *Bos bubalis* bulls stationed at Montgomery, Patna and Calcutta field artificial insemination centres were analysed by Bhattacharya and Prabhu.[22] The average volume of semen per collection varied according to species, breed, and within breed from animal to animal. The number of services required per conception was practically the same for both *Bos bubalis* and *Bos indicus*. A slightly higher fertility was obtained when 1.0 ml. of semen was used instead of 0.5 ml. and there was no decrease in fertility with semen upto 76 hours old. Data regarding semen characteristics of Tharparkar and Nagori bulls have been reported for the first time. Mukherjee and Bhattacharya [23] studied the seasonal variation in 'reaction time' and the semen characteristics of 6 Kaumauni hill bulls for 2 consecutive years. The quality of semen was distinctly superior in spring and inferior in autumn. The pH of semen showed highly significant monthly variations. Similar work on the semen of goat and ram have been reported by Shukla and Bhattacharya.[24,25]

Prabhu, Guha and Bhattacharya [26] replaced 30% of the total crude proteins in normal rations of Kaumauni hill bulls by blood meal for a period of 4 months and observed no significant difference in the various semen characteristics and reaction times of bulls fed normal and blood meal rations. Prabhu and Guha [27] found that with the exception of semen volume there was no significant difference in the semen characteristics and reaction time between bulls receiving and not receiving exercise just prior to collection.

While lack of exercise for long periods was detrimental to sperm production, an increase in the daily level of exercise from one to two hours produced no improvement in the quality of semen and reaction time.

Diagnosis, treatment and causes of lowered fertility in dairy cows were studied by Narayanan.[28] Prabhu and Bhattacharya [29] failed to detect any seasonal influence in the conception rates of cows artificially inseminated at Calcutta, Patna and Bangalore. Administration of thyroprotein to goats was found to increase the sperm concentration and the rate of fructolysis and decrease semen volume and initial fructose content.[30,31]

Cross Breeding for Milk Production

Analysis of production characteristics of Red Sindhi, F_1 Jersey X Red Sindhi and first, second and third back crosses to the Red Sindhi revealed no significant difference in the fat content of milk, length of calving interval, of survivability or young calves in the different groups.[32] The age at first calving decreased as relationship to the Jersey increased. Jersey X Red Sindhi cows in the first lactation produced 47% more butterfat than pure bred Red Sindhi. Sen, Lazarus, Rangaswamy and Anantakrishnan [33] have presented the results of cross breeding experiments of Red Sindhi, Sahiwal, and Hariana cows with Ayrshire bulls for milk production in Bangalore. Cross breds yield more milk, produce milk for more days, deliver their first calf at an earlier age and have longer productive life than their zebu ancestors. They also observed an increase in the percentage of incidence of foot and mouth disease and abortion in cows with an increase in the amount of Ayrshire blood. Both Ayrshire cows and bulls were found to degenerate under local conditions.

Management of Cattle

Narayan [34] has given an account of the different records that should be maintained by a dairy farmer. The performance of Gir breed in Madras State was reported by Murari.[35] The average milk yield, length of lactation and dry period were found to be 3800 lb., 277 days and 87 days respectively. A study of the performance of 63 Kangayam cows showed average milk yields of

2028 and 1,792 lbs. respectively in the first and fourth lactations.[36] Harbans Singh and Dave [37] studied the variations in the quantity and quality of milk from different quarters of the udder. Milking by individual teats or front and hind quarters in any order had practically no effect on the total milk yield. Hind quarters produced more milk than the front quarters. They reported higher yield from right hind teat and suggested its usefulness in judging dairy cattle. The milk yield of Sahiwal cows during 7 weeks' feeding period was not influenced by the feeding of 4 different types of fodders.[38] From the records of livestock Research Station, Hosur, Rajagopalan and Victor [39] estimated the sire index of 5 Red Sindhi bulls.

Kandaswamy Raju [40] made a study of the physical measurements of Kangayam cows in relation to their growth at Palayakottai cattle farm. There was a correlation between the minimum increase in weight (during 6th month) with the minimum increase in body measurement, while there was no correlation between the maximum increase in weight (4th month) and the maximum increase in the body measurements (2nd month). Anantakrishnan and Lazarus [41] studied the conditions affecting the birth weight of 1,048 Red Sindhi, 393 Gir and 364 Ayrshire X Sindhi calves. The average birth weight of calves from Red Sindhi, Gir and ½ Ayrshire X Sindhi cows were 45.6, 49.3 and 54 5 lb. respectively. Bull calves of all breeds were significantly heavier at birth than heifer calves. Cows having more than one gestation had heavier calves in the later than in the earlier gestations. Birth weight was affected by the breed of the sire, length of gestation and weight of the dam. The heaviest cows tended to produce the heaviest calves. Month of calving and the length of the preceding dry period had no effect on the birth weight of calves.

The effect of supplementing the ration of Sindhi and Gir heifers with iodinated casein on the growth and efficiency of food utilisation has been studied by Sen and co-workers.[42] During 28 weeks, animals receiving in the ration 2 gm. of iodinated casein/ 100 lbs. body weight grew at a rate approximately 43% and 55% lower according as they were below or above one year of age, than their respective controls. Pulse rate, respiration rate, body

temperature, body weight and haemoglobin content, as affected by environmental conditions, were studied at monthly intervals on 6 Kaumauni steers.[43] The most variable reaction with the change in air temperature was the respiration rate and this has been suggested to be one of the indices for assessing the heat tolerance of animals. From a record of 5,594 readings on 2,121 goats Bhatia and Sharma [44] have reported that the normal body temperature of goats in 4 different places of the Punjab varied from 99.4 to 101.18°F. The body temperature showed significant increase or decrease with a rise or fall of atmospheric temperature. The procedure for dehorning cattle has been given by Saha [45] and some work has also been done on the blood picture of bullocks,[46] goats and rams.[47]

DISEASES OF CATTLE

Kaushash [48] has presented some information about the geographical distribution and seasonal incidence of main contagious diseases in Madya Pradesh during 1936 to 1950. Highest incidence of Haemorrhagic Septicaemia, Black quarter and Anthrax was during the months of July and August. Foot and mouth disease was at its peak during February and March and the largest number of outbreaks of Rinderpest occurred during the months of March and September. A classification of these diseases according to the amount of rainfall in different places was also made. Rinderpest and anthrax are reported to be more common in the rice tract, haemorrhagic septicaemia in the cotton tract and foot and mouth disease in the cotton and rice tracts. Similar studies for 6 years for the Punjab were made by Tulsa Ram.[49]

(a) Foot and Mouth Disease

Seetharaman and Datta [50] have reported the various types of foot and mouth disease virus encountered in India. Besides the incidence of standard strains 'O', 'A', and 'C' and their variants, the occurrence of typical strain is also reported Valle 'O' type is preponderant (59.6%) in India.

(b) Carcinoma

Kulkarni [51] reported the incidence of cancer in bovine horns in the old State of Baroda. This disease was more common in the

Kankrej breed than in Gir or non-descript breeds. The author has suggested methods for preventing cancer. Lall [52] reported that during the years 1943-1952 there were 6,286 cases of horn cancer in Meerut circle. This disease mostly occurred in bullocks. It was stated to be common in large horned breeds and mostly occurred in the age group of 5 to 10 years and particularly in only one of the horns.

(c) Trypanomiasis

Ray and Bhaskaran [53] studied the efficacies of stilbamidine, HNO_3 and $HgCl_2$ tests as compared with that of complement fixation test for the detection of bovine surra. Stilbamidine (M & B 744) test detected the latent carriers among cattle earlier than complement fixation test. The treatment and control of bovine trypanomiasis due to *Trypanosoma evansi* was attempted by Ray and co workers.[54] Cattle clinically or experimentally infected with *T. evansi* were treated by Antrycide, methyl suiphate and pro-salt in concentrations varying from 3 mg. kg. without local or systemic reactions. The authors have recommended a dosage of 3 mg./kg. methyl sulphate with no relapse upto 3 months and with no deleterious effect on the milk yield of cows.

(d) Tuberculosis

The value of vole vaccination in the control of bovine tuberculosis was discussed by Lall and Sen.[55] It appears that mass vaccination neither with BCG nor with Vole bacillus will ever be attempted to any advantage for eradication of cattle tuberculosis, as the vaccination makes the animals allergic and a very useful method of detection of tuberculosis infection loses its effectiveness to a very great extent.

(e) Endometritis

Azizuddin [56] estimated the pH of the uterine discharges and amniotic fluid of normal and abnormal genital tract of cows. The pH of normal uterine and amniotic fluids were 7.08 and 7.22 respectively. The pH of uterine exudate in endometritis of bacterial origin, *viz.*, catarrhal, suppurative and atrophic endomet-

ritis were 7.50, 7.60 and 7.48 respectively, being near the optimum for bacterial growth. The pH of uterine fluid from animals with non-bacterial hyperplastic endometritis was acidic.

(f) Mastitis

Mohan [57] reported that over 100 lactating buffaloes and cows in organised dairy farms of Uttar Pradesh were found to be affected with suppurating mastitis during outbreaks of cow pox. The udder infection was usually associated with pox lesions on the teats. *C. pyrogenes* was isolated in almost pure cultures from the purulent udder secretions of several animals. About 46% of the cows and 31% of the buffaloes were found affected by Narayanan and Iya [58] in a survey of the incidence of mastitis in a dairy herd and the incidence increased with advancing lactation. Staphylococcal (β haemolytic) infections were frequently encountered amongst the cows while the incidence of streptococcal (both β haemolytic and non-haemolytic) infections was greater among buffaloes.

Basu and co-workers [59] observed that mange infection in livestock was definitely seasonal and was almost restricted to the months of October to March. *Sarcoptes scabei* and *Demodex folliculorum* were the common species of mites responsible for mange in domestic animals. A 2% solution of gamexane was effective for goats suffering from sarcoptic mange and 0.5% solution of DDT in kerosene for sheep suffering from psorptive mange. The same authors [60] studied the effect on milk yield of different insecticide sprays on cattle subject to attack by species of *Musca*, *Iyperosia*, *Stomoxys*, *Hippobosca*, *Hyalomma* and *Boophilus*. An average increase of 10.9% in milk yield was obtained over control with a spray containing pyrethrum and DDT.

2. MILK

Physical Properties

Dastur and coworkers [61] reported that the average freezing point of cow and buffalo milk were 0.545°C and 0.544°C respectively. Only 3% of the samples examined showed freezing point depression less than 0.530°C. A study of the phenomenon of

absorption of benzene vapours in milk showed that the adsorption was entirely due to the fat present, the magnitude of adsorption varying with the temperature, the size of the globule and the amount of fat contained.[62] On the basis of this property the authors have devised a method for the estimation of fat in milk.

Chemical Properties

The applicability of Richmond's formula for the calculation of total solids in milk has been verified by Ramachandran and co-workers[63] by using 1,529 individual and herd samples from cows and buffaloes. The calculated values were found to be both higher and lower than the gravimetric ones by upto ± 1.0. Only in 40% of the samples tested, the authors obtained agreement between the 2 methods within ± 0.25. Richmond's formula for buffalo milk also gave less satisfactory results in the hands of these authors. Dastur and co-workers[61] analysed lactose and chloride contents of cow and buffalo milk samples obtained from different parts of India. The average values for chloride and lactose for cow milk were 0.072% and 5.27% respectively and the corresponding values for buffalo milk were 0.060% and 5.47 % respectively. The mineral composition of milk was not influenced by different fodders, rich in some and poor in other elements.[64] Chanda[65] reported that the fat, solids-not-fat and chloride contents of the milk from cows suffering from mastitis were lower than the normal. The ratios of N to P in both milk and casein and the phosphatase activity were high in mastitis milk. Sarkar and co-workers[66] microbiologically assayed mixed proteins from cow, ewe, sow and mare milk for essential amino acids. The concentrations of the amino acids of such proteins were similar, although the arginine contents of sow and mare milk were somewhat higher. Between the milk proteins of different breeds of ewe there was no difference in the amino acid composition.

Bhatia[67] reported that the vitamin A content of cow ghee from the Punjab varied from 17.5 to 61.1 I.U./gm Narayanan, Anantakrishnan and Sen[68] have studied the effect of continued feeding of shark liver oil on the vitamin A content of milk of both cows and buffaloes. In a dry fodder regime of 18 weeks, administration of

100,000 to 200,000 I.U. of vitamin A/day increased the vitamin A content of milk appreciably and continued intake maintained the high level in both species. Chanda[69] studied the carotene and vitamin A content in the colostrum of cows and goats. The heifers secreted more vitamin A in their colostral fat than did the cows, and the cows at pasture produced more vitamin A in their colostral fat than did the cows on winter ration. There was no difference in the carotene content of the colostral fat in the first and later lactations in cows. The presence of β carotene in goat colostrum was demonstrated and vitamin A alcohol was found to be present in both cow and goat colostrum. The carotene and vitamin A contents of mastitis milk have been studied by Chanda.[65] In mastitis milk, the carotene content of the milkfat was high.

Basu[70] discussed the nutritive value of indigenous milk products. Nandi, Rajagopalan and De[71] prepared a vegetable milk by an admixture of 50% soya beans, 24% groundnut and 20% *ragi* malt with a suitable mineral mixture and reported that it was 88% as nutritive as cow milk. The availability of its Ca and P compared favourably well with that of cow milk.

Bacteriology of Milk

Chawan[72] has given an account of the spread of diseases through milk. The dye reducing activities of streptococci and lactobacilli using tetrazolium were studied in milk and nutrient broth by Laxminarayana and Iya.[73] They have also standardised the methods for the quantitative estimation of tetrazolium reduction. Sreenivasamurthy and co-workers[74] have estimated the vitamin B_{12} and vitamin B_{12a} contents of cow, buffalo and human milk with *Lactobacillus lactis Dorner*. The major part of the vitamin B_{12} activity of cow and buffalo milk was due to the hydroxy-cyano-cobalamin content; in human milk, however, the hydroxy-cyano-cobalamin content represented only about 50% of the total vitamin B_{12} activity. Sreenivasamurthy and collaborators[75] have developed a rapid method for the microbiological determination of riboflavin with a cell suspension of *Str. faecalis*. Microbiological assay conducted by Boman[76] showed that buffalo milk contained thiamine 0.50, riboflavin 1.02, niacin 2.60, biotin 0.13,

pantothenic acid 1.50, pyridoxine 3.25, para amino benzoic acid 26.76 and folic acid 5.50 μg/ml. Sreenivasamurthy and Iya [77] have examined the use of *L. plantarum* 89 as a test organism for the assay of riboflavin and the average values for riboflavin in the milk sera were 0.2 μg/ml. in human milk, 0.35 μg/ml. in mare milk and 0.5 μg/ml. in ass milk. The growth of *L. plantarum* was not affected by the presence of palmitate or oleate. Bhat, Broker and Iyer [78] studied the role of saffron and nutmeg in indigenous milk preparations. Aqueous extracts of saffron and nutmeg adversely affected the growth of spore forming bacilli. The activity of digestive enzymes was stimulated by saffron while nutmeg exerted a reverse effect on all but the gastric enzymes.

3. MILK PRODUCTS

Dahi

Iya and Laxminarayana [79] have described the method of making good *dahi* and the same authors [80] made an excellent review of the fermented milks prepared in the different parts of the world. Laxminarayana and co-workers [81] have made a qualitative survey of the types of *dahi* produced and marketed in different parts of India. Samples of *dahi* collected from North India were generally characterised by firm curd formation, sweet to mildly sour taste, agreeable flavour and with either a preponderance of streptococci or a balanced proportion of streptococci and lactobacilli. Yeasts were present in many samples and during monsoon both yeast and lactobacilli were more numerous. *Dahi* collected in summer and monsoon months were sour and showed gassiness and peptonisation. Samples from South India were usually poor in texture and sour to taste and had a preponderance of lactobacilli as well as yeasts. Wide variations in acidity, fat, protein and ash contents of *dahi* were observed. From over 500 samples of *dahi* collected from various parts of India, Laxminarayana and co-workers [82] classified 290 streptococci and 430 lactobacilli. The species of lactic acid bacteria occurring most commonly in *dahi* were *L. bulgaricus*, *S. thermophilus*, *S. faecalis*, *S. lactis*, *L. casei* and *L. plantarum* in the order of their frequency. Amongst the streptococci isolated from *dahi*, *S. thermophilus* and *S. faecalis*

were encountered most frequently while *L. bulgaricus* was the most commonly occurring lactobacillus in fermented milk. Some strains of streptococci and lactobacilli, capable of producing acid and of coagulating milk and of forming flavour compounds were isolated from samples of good quality *dahi* obtained in Calcutta, Benaras and a few other places in North India.

Ghee

Gur Bhagwant Singh [83] has given a description of making good ghee. Baliga [84] described the conditions of production and marketing of ghee in India and suggested methods for their improvement. Srinivasan, Mani and Ray [85] have studied the relative efficiency of water, saline and acidulated water as diluents to wash off on re-separation the non-fatty solids in cream to be used for the production of cream ghee. It was found that all the 3 diluents studied produced the maximum effect in washing when used at a level of 100% of the milk from which the cream was obtained and for maximum removal of non-fatty-solids, saline should have a concentration of 3% NaCl and the acidulated water a strength of 0.05% lactic acid.

Some work has also been done on the effect of method of preparation, temperature of heating, and antioxidants on the keeping quality of cow and buffalo ghee. Lalitha and Dastur [86] have found that ghee made by the *desi* method had a tendency to develop peroxides more rapidly and showed off-flavour at a higher peroxide value than creamery ghee. Butterfat melted by heating butter at 40, 65 and 115°C was found to keep well provided care was taken to remove the moisture and other impurities properly. Free fatty acids upto 1.5% were found to have no effect on the keeping quality of ghee. Ethyl gallate (0.02%) was very effective in checking oxidative spoilage but not the development of free fatty acids in ghee. Narayanan, Anantakrishnan and Sen [87] have studied the stability of vitamin A and carotene in cream ghee fortified with synthetic vitamin A. In 3 months, the extent of loss of vitamin A was below 3% in samples stored in glass, aluminium and tin containers and about 15% by the end of 6 months. Fortified cow ghee was more stable than fortified buffalo ghee. No vitamin A was found

in samples stored in mud pot containers at the end of 3 months' storage.

Guha, Guha and Ramnarain[88] determined the chemical characteristics of ghee available in wholesale and retail markets of Calcutta before and after war. Ghee from Uttar Pradesh was the best available in the market. Only 20% of the samples of ghee collected from retail shops could confirm to Agmark specifications and some measure for minimising the scope of adulteration have been recommended. Dewan, Kartha and Gulati[89] studied the use of indicator substances for the detection of hydrogenated oils present in ghee as adulterants. Ethyl ester of tyrosine was found to be non-toxic, stable, tasteless, not easily removable from hydrogenated oils and its presence in a concentration of 0.005% satisfied all the requirements of a tracer.

Khoa

The effect of abnormalities in milk supply on *khoa* production was studied by De and Ray.[90] Raw and heat processed milk allowed to age for varying periods was found to yield *khoa* of acceptable market quality provided the aging was not too prolonged. The use of a stabiliser to counteract curdling on boiling failed to make highly aged milk suitable for *khoa* making. The use of starch improved the quality of *khoa*. The shelf-life of *khoa* under room conditions was reported to vary between 7 to 9 days. Cold storage of *khoa* at 55°F or sugaring to the optimum content of 60% concentration-in-water of *khoa* augmented the life by 300 to 400%.

Other Milk Products

The method of making, conditions of coagulation, yield, storage property and uses of *chhana* are presented by Ray and De.[91] The composition, acid development on storage, food value and the uses of *chhana* whey for lactose manufacture are given by the same authors.[92] The method of production and the uses of casein and cottage cheese from skim milk have been given by other workers.[93,94] Subrahmanyan and co-workers[95] have evolved a method for the preparation of casein from deteriorated skim milk powder. The biological value of casein prepared from the

deteriorated milk powders was less than the biological value of casein obtained from fresh skim milk powder. The possible uses of recovered casein from spoilt milk powder for edible purposes, for plywood adhesive and for the preparation of medicinal protein foods are suggested.

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HUMAN PATHOLOGY

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The papers reviewed in this section show an encouraging increase in the analysis of disease as it is met with in India and in the application of the principles of pathology to the problems of this country. Increasing stress is being laid on chemical pathology and for that reason a section is devoted to this subject. For convenience the papers reviewed are grouped under ten headings.

1. Development abnormalities

Basu [1] has described a case of ectopic kidney observed in the dissecting room. This was in the usual position at the brim of the pelvis. He indicates the rarity of this condition by stating that it is the only case encountered in 1600 subjects which have passed through the dissecting hall in the Medical College, Calcutta.

Rajam *et al* [2] have shown how failure in the closure of the ectoderm of the median raphe may produce an epithelial lined canal in the scrotum. Such a canal is frequently the site of extra-urethral gonococcal infections.

Sen [3] has described from post-mortem specimens the various developmental anomalies of the lungs and bronchi. Jit [4] has described the development of mucous cysts in the human vermiform appendix associated with the growth of the lymphoid tissue.

Sundaraswarao [5] has investigated the Müllerian vestiges present in the testis in relation to the origin of benign epithelial tumours of the epididymis. Ninety-six testes from unselected autopsies were examined and six tumours of the epididymis were studied. From the autopsy study it was concluded that the appendices of the human testis and the epididymis are distinct. The former is covered with a layer of Müllerian epithelium closely resembling the fimbriated end of the fallopian tube. Cysts may develop in the epididymis from collections of Müllerian epithelium but also come from areas of serosal metaplasia. The six epididy-

mal tumours examined showed unmistakable evidence of their epithelial origin and one showed clearly Müllerian epithelium with a transition to an adenomatous structure. It was concluded that these tumours arise from Müllerian vestiges.

Bose [6] records a family of four children among whom three had osteogenesis imperfecta with multiple fractures. The fourth child had blue sclera but no fractures and x-rays of the bones showed no abnormality.

2. Infections

(*a*) *Viral infections* (see also serology)

Bhardwaj and Iyer [7] have investigated 661 cases of poliomyelitis developing in Bombay city from 1948-1953. There has been a seasonal incidence during the hot summer months (July to September). In recent years, however, there has been a shift to the earlier parts of the year. 91.8% of the total cases occurred in children under the age of five years. Only at the peak of the 1949 epidemic and at the peak of incidence in other years, were older persons involved. The greatest number of cases developed in the most densely populated parts of the city. It was thought that the movement of people and troops following the Burma campaign and the big influx of refugees after the declaration of independence and partition coupled with the strike for six weeks in the conservancy department in 1949 were important factors contributing to the 1949 epidemic.

Kalra [8] investigated an outbreak of influenza in an air force camp in Bangalore. The epidemic was confined to young trainees living in one group of barracks. The virus strain was cultured on embryonated eggs. Serological investigations and mouse lung adaptation tests were done. It was concluded that the infection was due to a strain of influenza—a primary virus.

Dogra[9] has described a method for the preservation of *Rabies virus* in the rabbit and the mouse brain by freeze drying which is more economical and lasting than maintaining the virus in 50% glycerine saline in a domestic refrigerator.

Kothare[10] has given an account of the clinical course and autopsy findings in eleven cases of encephalitis occurring in

Bombay. Attempts to isolate the virus proved unsuccessful.

Kerr[11] in a study of 576 human sera in Southern India has shown neutralizing antibodies to at least six different neurotropic viruses. None of these viruses have as yet been isolated in India. Specimens should be collected before the 4th day after the onset of fever for the acute phase specimen, and a month after the onset for the convalescent phase specimen. He uses yellow fever virus isolation as a model for the investigation of viral infections.

Misra[12] *et al* have made a careful survey of the available literature on tropical eosinophilia and have discussed their clinical, pathological and radiological findings in forty cases. In their experimental work they produced a rise in the eosinophil counts of mice and monkeys by injecting filtered sera of patients suffering from this condition, into the abdominal cavity or intravenously or intranasally. Work on rabbits had to be abandoned as the eosinophils were so readily confused with neutrophils. Agglutination tests against fowl's red cells were done with seven patients' sera. The haemagglutination titres were low, not reading more than 1 in 32. Inoculation of embryonated eggs with undiluted sera was inconclusive. These results suggested some support for the viral theory of the disease.

(*b*) *Bacterial infections* (see also lymph nodes)

Diphtheria

Tribedi *et al* [13] have undertaken a bacteriological and pathological study of diphtheria in Calcutta. Out of 252 cases investigated 224 were of the mitis type, 5 were gravis and 23 were atypical. Of the last 23, 13 were saccharose fermenters and of these 12 proved virulent on guinea pig inoculation. It was concluded that the gravis, intermedius and mitis groups were not always clear cut. Nine cases of mitis infection were studied after autopsy examination. These showed fibrinoid necrosis of the lymph follicles in the spleen and in the tonsils in nearly all cases. Toxic myocarditis was present in 7 cases and suprarenal haemorrhage in six. These findings closely corresponded to those of the Indian authors Das and Ghosal (Lancet, 1, 1410, 1953), that mitis infections in India produce more serious results than in England.

Staphylococci

Chakravarti *et al* [14] have studied the complexity of antigenic structure of staphylococci in an invsetigation of 400 strains including aureus, albus and citreus collected from both pathological and normal sites. The strains were tested with twelve monospecific absorbed antisera by a slide agglutination technique. Only 53.8% could be typed, 23% showed too many cross reactions and 23.2% were inagglutinable.

Typhoid

Modi and Dogra [15] consider that haemoculture is the only sure method of diagnosing the enteric group of organisms. In a series of 194 samples of blood from typhoid cases they found 70 positive in the first week, 95 in the second, 18 in the third and 11 in the fourth week of the disease. In paratyphoid A fever 28 samples were positive in the first week, 17 in the second week, 8 in the third week and 3 in the fourth week. Bacteraemia is not always of short duration. The clot is used for culture leaving the serum for a triple Widal test. Single triple Widal tests are of little diagnostic significance since a high titre may result from a recent attack preceding the present illness or a T.A.B. vaccination. In the absence of these factors a titre with "O" and "H" suspension above 1 : 250 and 1 : 125 respectively may be of significance. A rising titre even in absence of a positive haemoculture is of significance. Soman and Modi [16] have reviewed the diagnosis of enteric fever in Bombay over a ten-year period; B. typhosus was the commonest organism, B paratyphosus A was the next most common; there were no cases of paratyphosus B or C. The clot culture proved superior to whole blood culture. Single total and differential blood counts were of no diagnostic significance. In analysing their figures they find that one-third of the bacteriologically proved typhoid cases gave negative Widal reactions.

Gupta and Gupta [17] have compared the results obtained with agglutination tests for enteric disease using suspensions obtained from nine different laboratories—all but one in India. They showed a marked difference in the degree of agglutinability and titre with

these different suspensions when applied to the same serum. They showed that this could not be explained in terms of the number of organisms per million of the suspensions. Some suspensions with the same count per c.c. gave widely different degrees of agglutination and titre. It was thought that some of these differences may be due to blocking antibody.

Jhatakia and Yodh [18] have reported a case of meningitis produced by B. paratyphosus B, which was cultured from the C.S.F.

De and Bhattacharya [9] showed that the virulence of S. typhosus is not altered, as the resistance to chloramphenicol increases. When antibiotics are given in the middle of a course of inoculations of dead S. typhosus vaccine into experimental animals there is a fall in titre, which is not noted, if the drugs are given at the beginning of the course. Giving the drugs at a late stage in the disease is thought to account for some of the relapses.

Cholera

Sarkar and Tribedi [20] have shown the tremendous bactericidal power of vibrio cholera over B. coli by culturing vibrio cholera and B. coli together. Between the eighth and thirtieth days after inoculation no B. coli cultures could be isolated. This could not be completely explained in terms of altered pH.

De and Chatterjee [21] have produced fluid in an isolated loop of rabbits intestine into which they have injected living vibrio cholera. This fluid closely resembles cholera stool. The high albumin content suggests an alteration in intestinal capillary permeability.

Tuberculosis

Mhatre and Modi [22] have found that pathological material suspected of containing tubercle bacilli gives a greater number of positives on culture, if treated first with a 4% solution of sodium hydroxide than if treated with a hydrated salt of trisodium phosphate. The latter however inhibits contaminants and acid fast saphrophytes more satisfactorily.

(c) *Infections due to parasites* (see also serology)

Kant [23] has reported the presence of intestinal flukes in the

faeces of patients suffering from abdominal pain and dysentery, who live in the Saharsa District (North Bihar). The flukes were identified as F. buski. The source of the infection was found to be the tubers saruk, karkar, gheehul, chestnut and bhent found in pools and tanks. The cercaria encyst on these tubers which are eaten raw. That the intermediate hosts are the snails planorbus and leptocercii was demonstrated in three snails out of the 2,000 examined.

Lasrado and Swamy [24] describe a case with a hydatid cyst in the broad ligament. Twelve years previously a lump had developed in the right hypochondrium and this had been drained.

Reddy and Anguli [25] have reported 33 cases of hydatid disease from Madras over a twenty year period. Twelve of these were in the liver and only one in the lung. With increasing opportunities of examining the lung during life these numbers will almost certainly change in the future.

Ramdas [26] has reported three cases of guinea worm infection with a chronic encysted lesion developing around a calcified guinea worm. The abscess wall shows a foreign body reaction.

Gupta *et al* [27] have done some interesting work on the internal structure of L. donovani in its leishmania and its leptomonad forms. They show the presence of desoxy-ribo-nucleic acid in the nucleus and paranasal body of both forms. Ribose nucleic acid is present in the cytoplasm of the leptomonad forms. The methods of staining are described. This work opens up the new field of histochemistry in the investigation of cellular structure in parasites.

Dey and Kaur [28] have reported the first case of indigenous oriental sore encountered in Assam. A girl aged twelve years developed a red raised spot on the right side of her face. This ulcerated leishman's were isolated from the smears. The patient had been a resident of Assam for the past eight years.

In another paper [29] the same authors have reported ten cases of dermal leishmaniasis in Assam. In the nodular lesion the diagnosis can usually be made by smears. But it may be confused with leprosy and this is also true of the hypopigmented lesions in leishmaniasis where the parasite may be difficult to demonstrate. In the hypopigmented lesion the tissue microscopically shows a perivascular infiltration with macrophages and lymphocytes. In the

nodular lesion there is a clear zone at the junction of the dermis and epidermis. Below this are numerous macrophages in which there may be many parasites. Occasionally giant cells are seen. In some cases granulomatous tongue lesions are found.

Purandare [30] has reported a case of ileocolic intussusception in a male aged 35 in whom the cause appears to have been an amoebic colitis. The specimen was removed at operation but the necrotic condition of the bowel made a histological report inconclusive. The amoebic condition was not suspected until the patient died 34 days later. Amoebic ulceration of the colon and an amoebic abscess of the liver were found at autopsy.

(*d*) *Fungal infections*

While *rhinosporidisis* is a common condition in India, many aspects of this infection are still not known. Purandare and Deoras [31] have prepared a valuable report on 100 cases occurring in Bombay. There was only one female in the series. The nose was the commonest site (91). There were three cases in the conjunctivae, two in the lacrymal sac and one each on the lip, larynx, urethra and skin. The literature has been reviewed and from this it is pointed out that except for a few isolated reports all attempts at culture have failed, as have also attempts to inoculate animals. The method of transmission is not known. The majority of infections occurs in adults and contamination during swimming has been blamed. The histology of the condition has been carefully described and the clinical features discussed.

3. Serology (see also under bacterial infections)

Mehrotra and Gupta [32] have compared the results of 10,193 sera tested by the Wasserman test and the slide modification of Meinicke's test. They conclude that there is complete or partial agreement in 94.5% of cases and discrepant results in 5.5%. The slide test gives rise to fewer false positive reaction in leprosy but to some in tropical eosinophilia and malaria. The figures of agreement compare favourably with those when Wasserman and Kahn tests have been compared by other authorities. Meinicke's test is regarded as a useful routine screening procedure.

In a later paper [33] they have found in a series of 15,961 sera

that there was partial or complete agreement in 93.62% of cases. They found in addition to the above fewer false positives in malaria and none in pregnancy, Kala-azar and lupus erythematosis. There was however a higher percentage of false positives in cases of tuberculosis.

Gupta *et al* [34] have discussed the false positive serological reactions for syphilis in leprosy in 263 cases of clinical non-syphilitic cases of lepromatous and tuberculoid leprosy. The sera were tested with Wasserman reaction, Kahn test and Meinicke's and V.D.R.L. tests. The Meinicke's reaction gave the fewest false positives. The false positives were more frequent in lepromatous than in tuberculoid leprosy. Treatment had no influence on the serological reaction. In 43 out of the 50 sera giving false positive reactions, this became negative after heating the serum to 65°C. for four minutes.

Soman [35] has undertaken a clinical, serological and cultural study of three cases of Brucellosis. He was able to demonstrate that the goat incriminated in one case was infected but the agglutination test on the goat's milk was inconclusive. Rapid profuse growth was produced with chicken embryos.

4. Blood diseases

Chatterjee and Das Gupta [36] have made a careful analysis of 103 unselected cases of deficiency anaemias and conclude that 51.4% show evidence of dimorphic anaemia as defined by Trowel with evidence of deficiency in both antimegaloblastic factor and iron.

Singh *et al* [37] have examined the blood picture in 100 females of the upper middle class Punjabi families. These were either medical students or nurses. The majority were non-vegetarian on a mixed diet which included meat and eggs on alternate days or at least twice a week. Full details of the diet taken are given. A comparison is made between the figures and those previously reported in India and abroad. If each medical college in India were prepared to undertake a similar investigation valuable information could be obtained to standardise our "normal" values.

Such investigations are very routine but necessary. In most places, so much energy is devoted to the abnormal that there is not sufficient time to ascertain what the normal figures, in terms of diet, race and climate, are.

Venkataramiah and Krishna Rao [38] have examined one hundred medical students for *Rh subgroups* and have shown C antigen in 82%, C antigen in 67%, D antigen in 92% and E antigen in 27%. The sex of the students is not mentioned. In the distribution of the subgroups the authors comment on a striking resemblance to that found in Hebrews in Canada. Some suggestions in technique are made to avoid false positives.

Reddy [39] has described a useful formula for diluting fluid for counting platelets. The normal figures vary from 200,000 to 400,000 in his experience.

Heilig [40] has studied the problems connected with hookworm infections in Rajasthan. He has found an incidence of infection of 6.15% and 5.7% in all patients admitted to the medical wards of the Jaipur Mansing Medical College Hospital for the years 1949 and 1950. The infection is greatest among those who come from the vegetable growing areas and least in those who come from desert areas. He repeats the view which he has expressed before that there is often severe damage to heart muscle, but that the heart improves rapidly, following iron therapy and blood regeneration. He finds the anaemia to be always hypochromic and microcytic.

Bird [41] has investigated the presence of a dual receptor antibody structure in a random selection of Indian blood donors, who had not received transfusion. He has shown the presence of an anti A body linked to an anti B body and considers that this explains some cases of autoagglutination. These antibodies may not be separated by absorption.

5. Chemical pathology (see also liver diseases)

The increasing emphasis laid on the importance of chemical examination of the blood serum and other body fluids makes it important for investigations in this field to be summarised.

Ghose [42] has examined the serum calcium, phosphorus and

cholesterol in 32 pregnant patients living in Calcutta who showed symptoms of pre-eclampsia. There was a reduction in serum calcium and an increase in serum phosphorus and cholesterol. In another paper [43] he discusses the changes in the serum content of the same substance in 44 pregnant cases with seven grams % of haemoglobin or less in their blood. All these cases were observed late in pregnancy. There was a reduction in serum calcium in severe degrees of macrocytic anaemia but no significant alterations in the serum inorganic phosphorus. In macrocytic, normocytic and microcytic types of pregnancy anaemia, there is a fall in both free and ester fractions of serum cholesterol, which bears a direct relation to the fall in haemoglobin and red cell counts. The relation between the extrinsic and intrinsic factors and iron deficiency with the fall of blood calcium is discussed.

Mukherjee [44] has made a study of the plasma proteins in 93 cases of normal pregnancy attending an antenatal clinic in Calcutta. He showed a steady fall in plasma protein during pregnancy with a slight rise during the few weeks preceding labour. The fall is mainly due to a decrease in the concentration of serum albumin. There is a slight rise in fibrinogen. The average serum protein was 6.24%. The lowest level reached was a fall of 12.9% in the figure in early pregnancy. The fall may be partly accounted for by a rise in plasma volume. The author advances the view that rise in plasma fibrinogen conserves albumin which is required for the active growth of tissue in the foetus, uterus, placenta and breast.

Mukherjee and Mukherjee [45] have determined the serum iron content in healthy non-pregnant and healthy and anaemic pregnant women. They find that the serum iron falls in the second half of gestation but if adequate iron is available, the serum level can be maintained. Anaemias of pregnancy both micro and macrocytic are accompanied by a great reduction in the iron content of the serum. The same authors [46] have examined the iron binding capacity of plasma and iron saturation in 148 cases of anaemia in pregnancy. In this group there were 78 cases of deficiency, 58 cases of dimorphic and 12 cases of nutritional macrocytic anaemias. There was a control series of 70 cases of normal pregnancy and 50 non

pregnant healthy women. In normal pregnancy there is an increase in the iron binding capacity of plasma. The iron is normally carried by the β-1-globulin fraction and the amount of this globulin in the plasma can be estimated with a photoelectric colorimeter. At the same time there is a decrease in the iron saturation level of the plasma. In iron deficiency and dimorphic anaemias these changes are exaggerated. In nutritional macrocytic anaemias the iron binding capacity of the plasma was somewhat less than in normal pregnancy and considerably less than in iron deficiency anaemias.

Vyaghreswarudu [47] has reported eight cases of pseudo fractures or Milkman's syndrome. In some cases there was a low serum phosphorus and a raised alkaline phosphatase but the serum calcium was more or less normal. Histological examination of the bone from the affected area did not reveal any pathological change. The patients were all females between the ages of 38 and 47. Treatment with vitamins A and D and calcium was accompanied by improvement.

Murthi *et al*[48] report a case of advanced skeletal fluorosis with a periosteal reaction, increased density of the bones and marked mottling of the teeth. There was stiffness of the back and neurological evidence of pressure on the spinal cord.

Sen Gupta [49] *et al* have made electrophoretic pattern studies of the blood serum in Kala Azar. They have shown that in nine samples the most characteristic feature was a gross increase in gamma globulin associated with a decrease in albumin. In one case the only other positive serological test was the complement fixation. There were six negative aldehyde tests. In all these the presence of Leishman Donovan bodies in the bone marrow was confirmed.

6. Lymph node pathology

The lymph nodes have offered many difficulties to the pathologists and some interesting articles have been written on various aspects of disease as it affects them.

Goyal and Samuel [50] have analysed 160 lymph node biopsies. 107 of these were tuberculous and such nodes were most common

in the second and third decades, in the cervical region and in females. After radiotherapy increased fibrosis and reticular fibres could be demonstrated.

Seth *et al* [51] in a review of 75 lymph nodes diagnosed both clinically and histologically as tuberculous have shown a very wide variable destruction of the reticulum fibres. In 38 nodes there was complete destruction of the reticulum in the caseous areas. In 12 cases there was only partial destruction but in 25 cases there was a complete preservation of the reticulum framework. The reticulum fibres were continuous with those at the periphery of caseous tissue around and in between the epithelioid cells. The reticulum was found to be condensed around the caseous areas. One surprising feature was to find the reticulum preserved in two areas of caseation in which calcification had taken place.

Furniss [52] has discussed the pathology of lymph node in leprosy in a thoughtful paper. 60 nodes were examined. The majority were from biopsy but 10 were from necropsies. In lepromatous cases the nodes were infiltrated with large pleomorphic cells with pale vesicular nuclei and foamy or uniformly eosinophilic cytoplasm. In some cases there was little normal node present; in other cases the infiltration was in scattered foci. Giant cells were present but were never of the classical Langhans type. The giant cells contained large vacuoles. The cells resembling lepra cells, were packed with acid fast bacilli. Silver impregnation did not show any increase of reticulum. There was no caseation without tuberculosis. In neural or tuberculoid cases none of these changes were found. Cases suspected of tuberculosis had this suspicion confirmed by guinea pig inoculation or by other necropsy evidence.

Tribedi and Mallick [53] have reported their experiences with 510 cases of lymph node biopsy. 56% of the cases was due to tuberculosis. The incidence in the inguinal region was higher than in the axilla-one in every eleven cases occurring in the former site. The source of infection in three cases was the penis, the scrotum and lupus of the foot. Breast infection was the commonest cause of axillary gland involvement. In one case there was a

combined malignant and tubercular involvement of a lymph node. Reticulosarcoma and lymphosarcoma occurred with about equal frequency. In nine cases of enlargement of glands following B. C. G. vaccination, there was a histological picture resembling tuberculosis. There were seven cases of Boeck's sarcoid. There were only seven cases of Hodgkin's disease. Chronic lymphatic leukaemia was diagnosed in only two cases.

De Sa [54] in an interesting paper has examined the scars on the legs and arms of children who had tuberculous adenitis in the groin or axilla. There were 9 positive results as judged by the histological picture out of 12 excised scars. Tubercle bacilli could not be demonstrated but further attempts by the concentration method are being made. Surgical excision of the suspected scar is often followed by dramatic improvement in the tuberculous lymphadenitis.

7. Liver diseases

The pathology of liver diseases has been so widely discussed and is of such importance in India that a special section seems advisable.

Das Gupta and Singh [55] have investigated the liver function tests in 43 cases of portal cirrhosis and 21 normal individuals with histological confirmation of the diagnosis in some of the cases. They found jaundice present in only 14 of the 43 cases with the highest serum bilirubin at a level of 5.9 mgm. In almost all cases of cirrhosis the globulin was raised above 3 g. with a corresponding fall in albumin and a reversal of the albumin/globulin ratio. The cholesterol flocculation test was normal in only seven cases, while the thymol turbidity and flocculation were normal in only two cases.

Arora and Wahi [56] have made further contributions to their studies of liver function. They have prepared charts covering the following tests: serum bilirubin, total plasma proteins, albumin, globulin and fibrinogen estimations, thymol turbidity, thymol flocculation, colloidal gold flocculation, cephalin cholesterol flocculation, bromsulphalein retention, prothrombin time and urine urobilinogen. The normals for these figures all fall on the

same horizontal line. Variations in disease will assume a pattern if the levels of the figures for the various tests are joined. Patterns of response are described for portal cirrhosis, epidemic hepatitis, acute malaria, chronic malaria and congestive heart failure. However these graphs were not considered specific enough for diagnosis and were only used as an indication of liver damage.

Patwardhan *et al* [57] have undertaken some extremely interesting histochemical studies on liver damage in 100 rats poisoned with carbon tetrachloride given by a single intraperitonea[l] injection. The animals were killed by stunning 6, 12, 18, 24, 72, 96 and 120 hours after the injection. The liver was examined for fat, glycogen, nucleoprotein, alkaline phosphatase, and acid phosphatase by histochemical methods, and total nitrogen, glutatathione and methionine by biochemical analysis. These examinations showed profound biochemical and histochemical changes before there were signs of structural damage as seen in routine H. and E. stained sections. The earliest damage is a reduction in cytoplasmic basophilia and alkaline phosphatase in the centre lobular zones. This was found six hours after injection of carbon tetrachloride. At eighteen hours balloon cells in the midzone were a constant feature and it was suggested that these, by interfering with the circulation to the centre lobular cells, produced necrosis. Between 12 and 18 hours there was an increase in the peripheral alkaline phosphatase. This was thought to be due to interference with the circulation.

Wahi and Arora [58] have studied 88 cases of epidemic hepatitis in an out-break which developed in Uthar Pradesh from May to September 1949. They comment on the fact that the majority of fulminant fatal cases occurred in pregnant women. Four foetuses, delivered from mothers, who died were autopsied. One of these showed changes characteristic of epidemic hepatitis. They believe from their investigations that when epidemic hepatitis becomes chronic it results in postnecrotic scarring and not in diffuse hepatic necrosis.

Heilig and Sharma [59] have described two cases of severe infective hepatitis which were aroused from deep hepatic coma by the use of terramycin. They have also described the use of

aureomycin in less severe cases. The course of the disease was followed with biochemical tests but unfortunately no liver biopsies were done.

Balasubrahmanyan [60] has investigated the ground substance present in the fibrous tissue which develops in experimental rat liver cirrhosis following poisoning with carbon tetrachloride. In the early stages an interfibrillary substance which stains with periodic acid (Schiff method) was found. This was removed by hyaluronidase. It decreased in amount as the fibrous tissue became older.

Tribedi and Chandra [61] have reported an autopsy case with sharply localised areas of liver necrosis which could easily have been missed by liver biopsy. They emphasise the danger in drawing too many conclusions from a single liver biopsy examination.

8. Circulatory disturbances

Reddy [62] has reported a case of a fusiform pulmonary aneurysm in a male patient aged 25 which had ruptured into the left bronchus. Chaudhuri and Chakravarty [63] have carried out experimental work on rats and monkeys to show the relation of feeding with argemone to epidemic dropsy. The rats developed marked congestion in the heart, kidneys, and liver, after a total dose of 5 cc. spread over two months. Monkeys developed varying degrees of congestion when doses were combined over a period of six months. Mehrotra *et al* [64] have reported a case of primary pulmonary arteriosclerosis in a male patient aged 24. There was right ventricular dilatation and hypertrophy, intimal plaques in the pulmonary artery and marked congestion of the liver. Sections of the lungs showed marked intimal thickening of the medium sized and small pulmonary vessels. In some cases organising thrombi undergoing recanalisation were seen.

Betts and Thomas [65] have reported a case of patent ductus arteriosus with endocarditis which was treated successfully by ligation. An organising infarct was removed at the time of operation from the left upper lobe of the liver.

Mehrotra [66] has investigated the changes which follow ligation of the carotid, femoral and saphenous arteries and the femoral and

saphenous veins of rats. The animals were killed at intervals of 1, 2, 3, 5, 7, 9, 14 and 28 days and at intervals up to thirteen months after ligature. In the arteries there was a proliferation of endothelial cells which gradually increased in thickness until after 90 days the lumen was obliterated. This was accompanied by atrophy of the media. In the veins endothelial and fibroblastic proliferation occurred easily and this was followed by recanalization and the formation of a new vein. In the arteries with their thick muscular walls there was much slower repair.

Mehrotra[67] has also investigated the changes in the vesical circulation following distension of the bladder. He has shown by transillumination that complete urethral obstruction leads to slowing of the circulation and ultimately stasis and haemorrhages. This is followed by necrosis of the epithelioid lining and acute inflammatory celluar infiltration. Such chances render the bladder wall more vulnerable to infection. Similar changes occur in the kidney, pelvis and renal parenchyma.

De[68] has given a most valuable and critical analysis of the papers dealing with the corticomedullary diversion of blood in the kidney and its possible relation to acute renal failure. Trueta's concept, gained from animal experimentation, has been applied to man but the reviewer councils against a complete application of the theory. He discusses two cases of bilateral cortical necrosis which developed in cholera and includes other personal observations on this condition. There is an adequate and well selected bibliography.

9. Tumours

Under this head are grouped the very widely diverse group of tumours reported during the year. There is still insufficient recording of interesting or unusual tumours and incomplete knowledge of tumour growth and incidence in India.

Gupta[69] has reported a case with multiple plasmacytomas involving pelvic bones and the skull in a male patient aged forty, who was first thought to have osteomyelitis and on X-ray examination was diagnosed as osteoclastoma. Biopsy revealed the true nature of the tumour. Gupta[70] has also reported three cases of

extramedullary plasmacytomas. The first was found in the palate of an 80 year old female. The second in a male patient aged 35 with a tumour in the wall of the caecum. Examination of the urine following operation showed the presence of Bence-Jones protein. The third case had a swelling on the inner surface of the right cheek adherent to the maxilla. Bence-Jones protein was absent in the first and last cases.

Mangalik and Wahal [71] report a case of bilateral sympathicogonioma of the suprarenals developing in a seven year old male child. The tumour arose from the right suprarenal while the left suprarenal had either been infiltrated or was the site of a second tumour. The whole mass was fused together and with the adherent viscera weighed 3000 g. There was spread to the regional lymph nodes and via the blood stream, spread to the liver. Tumour emboli were found in the vessels of the liver and kidney. The tumour cells showed some rosettee arrangement.

Singh and Singh [72] describe a case of malignant synovioma in a man aged 60. This was present on the medial side of the left thigh just above the knee. The histological picture showed a pseudoepithelial structure with gelatinous secretion which superficially resembled adenocarcinoma. The tumour recurred locally three times during a period of three and half years and finally developed metastases in the lungs.

Patil [73] has studied eleven cases of chorion carcinoma in relation to gonadotrophic hormones in the urine as indicated by Friedman's test. She considers that the test should be done as a routine on all cases of vesicular mole and in the follow up of such cases. However chorion carcinoma may exist even with a negative Friedman's test. Lutein cysts are present in 25% of cases. If these are not removed at the operation (since they store gonadotrophic hormones) positive pregnancy tests may persist after removal of the original tumour. The use of the test with blood serum and cerebrospinal fluid is discussed.

Wahi and Gupta [74] have published their experience with the smear diagnosis on 400 cases of suspected carcinoma of the uterus. In all but 51 cases the diagnosis was confirmed by histological examination. There were 5 false positives and 9 false negatives

with an accuracy on the total of 96%.

Lawande [75] discusses the findings in a study of 50 cases in whom pleural and peritoneal fluids were examined for malignant cells. In the nineteen positive cases the clinical diagnosis had been made in eleven. Out of the thirty-one cases in which malignancy was not detected twelve were definitely malignant. These latter cases are explained by the fact that the tumour had not reached the serosal surface but had produced an infarct like effusion by obstructing the circulation.

Gault *et al* [76] have analysed 110 consecutive cases of intracranial space occupying lesions diagnosed by pathological examination at Vellore, South India. The incidence of the different types of lesion corresponds closely to that met with in series of cases from other countries but the percentage of gliomas is lower and that of tuberculomas (18%) and parasitic infections (5.4%) is higher. Glioblastomas are considered an anaplastic variant of astrocytomas. The medulloblastoma is a true embryonic tumour. Two cysts of the corpus callosum and an unusual dermoid with subarachnoid spread of the cyst contents are reported. One case of retinoblastoma with intracranial spread and metastatic deposits in the liver and lungs is discussed. Chandy [77] has made a clinicopathological survey of the first hundred verified brain tumour reported in India.

Thomas [78] has made a study of the blood pattern which is formed when three or four drops of blood are allowed to dry on a slide. Bolen claimed that a specific pattern developed with the blood from cases of malignancy. 525 tests were done of which 307 gave a positive pattern. The patients with positive and negative patterns were analysed in terms of their clinical conditions. The test was found to be accurate in 90.2% of cases. Extensive liver disease, intravenous administration of saline, pregnancy and blood transfusions were found to give false positive reactions.

Wahal *et al* [79] report two cases of a struma ovarii. In one case the tumour was bilateral; on the right side there was in addition a large sized serous cyst. In the second case the thyroid tissue was associated with a pseudomucinous cystadenoma. The authors consider that the term struma overii should be restricted to

those tumours in which one fails to find any teratomatous tissue present.

Ranadive[80] has undertaken a careful study with a good review of the literature of the factors inducing carcinoma in mice. She used two female strains one of which was highly susceptible and the other a highly cancer resistant strain. They were foster nursed and ovariectomised at birth and received the same dose of hormones at maturity. She concluded that there was little genetic susceptibility and that hormonal stimulation (with separate groups stimulated by oestradiol and testosterone) only affected the ductal pattern. The milk born factor was the most important etiological factor and this was responsible for acinar proliferation indicative of abnormal growth.

Govinda Reddy [81] has reviewed 90 giant cell tumours of bone diagnosed in the Pathology Department of the Madras Medical College, 1938-1948. These tumours formed nearly one-third of all the bone tumours met with over this period. The age incidence corresponded closely to that found in other countries There was a greater incidence of tumour in the small bones of the hand and foot as compared with other series. Open biopsy was advocated for diagnosis. The tumours were classified into three grades according to the number, size and type of giant cells and the nature of the stroma, second grade tumours were liable to recur and third grade tumours were frankly malignant from the beginning. The author considered that prolonged irradiation may increase the agressiveness of the lesion. Grade one tumours require only curettage and filling of the cavity with bone chips.

Nigam [82] reports a series of 19 cases of tumours of peripheral nerves treated by him in the last three years and discusses two cases of perineural fibroblastoma in detail.

Sirsat [83] has prepared an excellent review of 121 cases of salivary tumours encountered at the Tata Memorial Hospital over a period of twelve years. He has divided the tumours into five groups, a mixed type, papillary lymphadenomas, mucoepidermoid tumours (both benign and malignant), cylindromas and carcinomas. The mixed tumours (68) formed the largest group. Ten of these tumours were found in the palate and one in the lip. The parotid

gland was the site of origin of 70% of the mixed tumours. The more recently described mucoepidermoid tumours are shown by photomicrographs to be both innocent and malignant. Twelve per cent of the total tumours were cylindromas. Carcinomas formed 18.2% most of these were in the parotid; the majority were from patients between forty and sixty years of age. There were three cases of adenolymphoma.

Reddy [84] reports a case of haemangioendothelioma of the spleen and discusses the rarity of all forms of primary splenic neoplasm.

Sarkar [85] reports a case of intramedullary lipoma of the spinal cord which was exposed at operation and diagnosed by biopsy but could not be completely removed.

Sundaraswarao and Sundararaman [86] report a large retroperitoneal fibroma measuring $18\frac{1}{2} \times 17 \times 4$ inches at operation; this was thought to be malignant but at autopsy the tumour was easily shelled out.

Aikat and Balakrishna Rao [87] describe a case of the rare "large cell small acinar Langhan's adenoma of the thyroid." There were islands of foetal adenoma present and this suggested a possible origin for the tumour.

Das [88] presents a case of retroperitoneal dermoid lined by stratified squamous epithelium and containing hair and sebacious secretion which occurred in a male patient aged 22. The author concludes that these tumours are one-sided teratomas.

Roy and Chakravarty [89] report two cases with dysgerminoma ovarii occurring in females aged 13 and 20 in whom the uterus appeared to be underdeveloped.

Srivastava [90] in a discussion of carcinoma of the oral cavity and lower jaw met with in the Agra Medical College finds that most cases came from the Manipuri district where a special kind of tobacco mixed with areca-nut is used for chewing. He considers that the mixture of tobacco and lime is also a causal factor. He did not find the grading of tumours of much help in indicating their invasive properties with involvement of bone and lymph nodes.

Amesur [91] reports a case of multiple intussusceptions of the small bowel in a female patient aged 45 who had twelve adenomatous

polyps in the small intestine and two polyps in the large intestine.

Kumar *et al* [92] have described two cases of stromal endometriosus. In one case the condition was benign and was associated with bilateral thecomas. In the second patient histological examination showed malignant change. Patients with this condition have usually reached the second half of their reproductive life and excessive vaginal bleeding has usually followed a long period of infertility. The authors discuss the theories of the etiology of this unusual condition.

Rajam *et al*[93] have described three cases of erythroplasia of the penis which is a form of carcinoma *in situ*. This starts as a painless persistant shiny brilliant red patch on the mucous membrane of the glans penis and the prepuce in middle aged uncircumcised men.

Bhattacharya[94] *et al* have reported 15 cases in which radioactive phosphorus has been injected intravenously in cases of breast tumours. They claim that the estimation of beta activity with a radioisotone over the affected area makes it possible to distinguish between benign and malignant neoplasms.

Chakravarty *et al*[95] report a case of retroperitoneal liposarcoma in a male patient aged 60. At autopsy secondaries were demonstrated in the right lung and the 12th thoracic vertebra. The tumour weighed 31 pounds and filled the abdominal cavity.

10. Miscellaneous

Some of the most interesting papers written during the year are those by Reddy and Ratnavati [96] on their experience with the autopsy findings in still births and neonatal deaths. In a series of 162 autopsies they found that 47% of the infants were premature (less than five pounds in weight at birth). In only eight cases was no cause for death found. Maternal complications such as toxaemias of pregnancy, accidental haemorrhage, placenta praevia, prolonged labour, rupture of the uterus and maternal syphilis accounted for 38% of the mortality. In only nine cases could the method of delivery be blamed for the death. Causes of death were asphyxia, pneumonias, intracranial, renal and adrenal haemorrhages, congenital malformations and maternal syphilis in

this order of frequency. Histological examination showed examples of renal vein thrombosis, pulmonary hyaline membrane, vernix squames and congenital syphilis as causes of death. Two cases of hydrops foetalis unassociated with erythr oblastosis were found. In a further paper [97] they analyse the pulmonary lesions in 180 still births and neonatal deaths. These accounted for 32.5% of the neonatal deaths but only 3.7% of the still births. Only in two cases was the clinical condition recognisable. It was considered that pulmonary haemorrhage predisposes to infection. 55½% of infants with pulmonary infection had premature deliveries. One case with hyaline membrane is discussed in detail and the view expressed that it results from aspiration of vernix caseosa. Another paper [98] describes five cases of congenital syphilis among 106 still births and one neonatal death among 56 neonatal deaths. In one patient there was marked fibrosis of the placenta and one child had a gumma in the lung. The last paper [99] of this series refers to the frequency with which haemorrhages are found in the lungs, medulla of the kidneys and adrenals, liver and subdural and subarachnoid spaces in cases with obstetrical and clinical evidence of asphyxia. The capillaries of the newborn are found to be fragile and very liable to rupture. Subcapsular haemorrhages in the liver were frequently found. In some cases there was haemorrhage into the thymus. Pulmonary oedema and bronchopneumonia were invariably associated with deaths due to intracranial haemorrhage which recalls the interesting observations of Cameron and De. (J. Path and Bact; 1949, **61**, 298) Work of this kind should be repeated in other medical centres as there is insufficient knowledge about the cause of many neonatal deaths and stillborn babies.

Reddy [100] has pointed out how valuable medicolegal postmortems can be, in revealing unsuspected pathological conditions. Diffuse bilateral bronchiectasis was found in a male child aged two. He comments on the persistence of epithelium over areas of inflammation in the wall of the bronchus.

Thomas *et al* [101] have reported two cases of female patients with congenital oesophagobranchial fistulae aged thirteen and fourteen. Both patients had bronchiectasis.

Sobti [102] has reported two cases of swallowed foreign bodies

which were removed from the stomach. In one case a long twig of wood used in cleaning the teeth was swallowed two months previously; in the other case a sewing needle swallowed three days previously had produced a perforation of the stomach wall near the pylorus which had been sealed off by omentum. Vasudevan [103] has recorded a patient who swallowed two handfuls of river sand one month before operation. The sand had remained in the stomach and ulceration of the mucosa had occurred.

Reddy [104] has analysed 28 cases of head injury seen at autopsy and he points out that gross intracranial damage can exist with an intact skull; laceration and haemorrhage of the brain stem often results from the indirect effect of compression leading to cerebral oedema and venous obstruction. 68½% of the cases showed pulmonary lesions with varying grades of haemorrhage and oedema, and in more servere cases pneumonia.

Wahi and Chandra [105] have reported a case of ichthyosis uteri with replacement of the endometrial tissue by large sheets of cornifying stratified squamous epithelium.

Vyaghreswarudu [106] has described the only case of Paget's disease seen in the orthopaedic department of the Madras General Hospital in the last five years. The patient was a male aged 39. X-ray showed thickening of the bones of the pelvis, tibia and skull. The Wassermann and Kahn tests were negative. Unfortunately the patient would not allow a biopsy examination.

Tribedi and Roy [107] have reported a case of primary systemic amyloidosis in a male patient aged 36 At autopsy the liver weighed 2720 g. and the spleen 1640 g. Amyloid material staining with Methyl violet was demonstrated in the medium small sized arteries of the heart, in the middle coat of the arteries in the submucosa of the stomach, in the vessels of the spleen, kidney and pancreas and prostate. The liver showed extensive deposits of amyloid with atrophy of the liver cells. There was an extensive deposit of amyloid in the zona glomerulosa of the adrenals. No infective causative factor could be found.

Cameron and Mehrotra [108] have investigated the regeneration of tissues in rats following injury by freezing with carbon dioxide snow. They show that necrotic cells are removed and replaced by

reparative tissues at different rates. Regeneration occurs most rapidly in the skin of the ear, the liver, spleen, pancreas and voluntary muscle. It occurs less rapidly in the adrenal and most slowly in the fatty omentum, kidney and testis. If more rapidly regenerating tissue is transplanted, after injury, into the omentum or testis of the same animal, there is retarded repair. When the testis has undergone atrophic changes repair following injury is accelerated. These authors have opened a new and interesting field for the investigation of injured cells and their environment.

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GENERAL MICROBIOLOGY INCLUDING ANTIBIOTICS

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During the year under review, the field of antibiotics has continued to attract the attention of large number of investigators, while interest in industrial fermentation seems to have waned. Those working on antibiotics had the opportunity of exchanging views and notes during the Symposium on Antibacterial substances held under the auspices of the Pharmaceuticals and Drugs Committee of the Council of Scientific and Industrial Research from 26th to 28th March 1953 at St. Xavier's College, Bombay. Sir Alexander Fleming, the discoverer of Penicillin, participated at the Symposium. As many as 21 papers were presented and the proceedings have been published in November and December issues of the Indian Journal of Pharmacy.[1]

The work under review has been classified under six heads : (i) Antibiotics, (ii) Industrial fermentation, (iii) Immunological studies, (iv) Cytogenetics, (v) Enzymes of micro-organisms, and (vi) General Microbiology.

I. Antibiotics

Gupta, Dudani and Shrivastava [2] have isolated an aerobic spore forming bacillus from garden soil, the broth filtrate of which possessed antibacterial activity against both gram positive and gram negative bacteria, particularly the latter group.

Gangadharan *et al* [3] have continued their studies on the antitubercular activity of Sesamin which they had reported the previous year.

Krishna Murthy, and Rao [4] have isolated and studied a new crystalline pigment Moreollin, from the seed coat of *Garcinia morella*. Moreollin was effective against *Micrococcus pyogenes* and *Mycobacterium phlei* in a dilution of 1 : 10,000 at pH 7.2, but was less active against E. coli.

Bhat and Broker [5] have tried the *in vitro* action of some plant

extracts on pathogenic staphylococci. The extracts delayed the coagulase producing capacity and the mannitol fermenting ability of the organisms but had no effect on their gelatine liquefaction and haemolytic activities.

Broker, Gajjar and Bhat [6] have determined the phagocytic response in guinea pigs to extracts of leaves of *Murraya Koenigii* and Arecanut. Both the extracts had favourable effect in *in vivo* studies and were non-toxic.

Baichwal, Khorana and Vangikar [7] have measured the phenol co-efficients of 38 halogen and other derivatives of β-Naphthol. Of these, 3-iodo-β-naphthol had bactericidal action against *B. typhosus* at a concentration as low as 8 mg. per c.c.

Lewis [8] has compared the effects produced by Streptomycin and Isoniazid.

Majumder and Sen [9] have developed what they claim to be a new method for isolating antagonistic organisms from the soil.

II. Industrial fermentation

Lewis and Johar [10] have investigated the spore size and appearance of *Aspergillus niger* in relation to citric acid yield.

Lulla and Johar [11] have tried *Penicillium notatum* as a source of fungal pectinase. The amount of pectinase produced was highest in a lucerne medium.

Lobo, *et al* [12] have developed a suitable medium for isolating yeasts from such natural habitats as the intestinal tract of normal human beings, ripening fruits, seawater, etc.

III. Immunological studies

Banerji and Mukherjee [13] have determined the nutritional requirements of two strains of *Pasteurella septica*. They found that pantothenic acid and nicotinamide were essential for both the strains while thiamine and riboflavin were dispensable.

Seal [14,18] has published a number of papers on *Pasteurella pestis*, *P. pseudotuberculosis* and their variants. In his first paper,[14] he has reported the growth, morphology, salt stability and biochemical reactions. By agglutination and cross agglutination [15] studies among the strains of *Pasteurella*, he has found that

pseudotuberculosis has three antigens, one of them probably in common with the somatic antigen of *P. pestis* strain but did not contain the protective antigen of the latter. By serological and precipitin studies [16] he was able to show that the soluble antigen of *P. pestis* insoluble in 1/3 sodium sulphate saturation was the protective antigen. This antigen fraction also had the complement fixing and immunogenic properties [17] of *P. pestis*.

Seal [18] has published a note on the methods for the differentiation of *Pasteurella pestis* and *P. pseudotuberculosis*.

Cohly, Giri and Rao [19] have determined the electrophoretic pattern of human serum proteins in small-pox. Sen Gupta, *et al* [20] have carried out a similar study of human serum in Kala-azar. In both the cases there was an increase in gamma-globulin fraction at the expense of the albumin fraction. Lahiri and Rao [21] have determined the electrophoretic pattern of polyvalent anti-snake-venom horse serum and its pseudoglobulin fraction.

More and Rao [22] have found that the extraction of lipids from diphtheria antitoxic pseudoglobulin resulted in the insolubalization of the alpha-globulin component. There was no loss of antitoxic titre after lipid extraction but on the other hand, there was an increase in purity due to the removal of the non-antitoxic alpha-globulin.

Lahiri [23] has claimed a high antigenicity for his new diphtheria prophylactic (NAFT) in field trials.

IV. Cytogenetics

Subramaniam [24] has presented photomicrographs to illustrate normal and abnormal mitosis in a strain of tetraploid yeast. The origin of diploids, triploids, pentaploids and hexaploids from tetraploids are described based on mitotic findings. In a later paper, Subramaniam *et el* [25] have given evidence for formation of a triploid yeast from a diploid by an extra splitting of one of the two chromosomes. Royan [26] has observed spontaneously formed autotetraploids arising from a normal diploid culture.

Prahlada Rao and Subramaniam [27] have observed differences in cytological pictures of yeast cells taken from a 24-hour agar slant and that from actively proliferating cells in well aerated

media. The evidence showed that the varying number of bodies seen represented an ascending grade of endopolyploidy.

Duraiswami [28] has found no close correlation between mitosis and cytokinesis in yeast. This he explains on the basis of the peculiar mode of division of yeast cells probably due to the existence of cytoplasmic streaming movements during cell division.

Subramaniam and Ranganathan [29] are of the opinion that yeasts of the Torula type which do not sporulate need not necessarily be haploid.

Mundkur [30] has measured the mean diameter of yeast nuclei using freeze-dried preparations in preference to conventional chemical fixatives which might cause shrinkage of nuclei. He finds that the mean nuclear diameter is a measure of the degree of ploidity.

Mundkur [31] has measured the number of mitochondria in yeast using a phase-contrast microscope. He finds an occasional clone deficient in mitochondria but observes that the normal complement may be restored on outcrossing to normal cells.

Mitra [32] has studied the mutagenic action of chrysene on a diploid brewery yeast. A tetraploid was obtained which fermented sugar solutions faster than the parent diploid.

V. Enzymes of Micro-organisms

Krishna Murti and co-workers [33-34] have continued their study of enzyme inhibition in relation to drug action. They have tried the action of Dihydrostreptomycin, Terramycin, Aureomycin, Chloromycetin, and Penicillin on alkaline phosphatase [33] and on liver arginase.[34] Datta and Basu [35] have studied the action of quinine on enzymes of the tricarboxylic acid cycle.

Shrivastava and co-workers [36-40] have studied the enzymic make-up of *Vibrio cholerae*. They have studied the gelatinase activity,[36] aspartic acid deaminase,[37] oxidative enzymes,[38] dehydrogenases,[39] and enzymatic hydrolysis of glutathione.[40]

Arora, Dudani and Krishna Murti [41] have studied arylsulphatase in certain pathogenic bacteria.

VI. General Microbiology

Khambata and Bhat [42-43] have isolated six new strains of

oxalate-decomposing bacteria, *Pseudomonas oxalaticus*, from the intestine of Indian earthworms. They have studied their cultural characteristics and nutrition.

Pandit, Iyer and Bhat [44] have examined Indian Cottage cheese and found the presence of various *Bacilli* and *Cocci* as well as species of *Aspergillus* and *Penicillium*. They have reported that juice of fresh sour limes has promising preservative qualities for cottage cheese.

Pavri and Bhat [45] have studied the microorganisms associated with the leaves of *Pongamia glabra*. They have isolated various strains of *Pseudomonas* species and *Micrococcus citreus*. They have suggested that insects as well as bacteria may symbiotically cause galls to form on the leaves.

Bhat and Albuquerque [46] have investigated the micro-organisms associated with the fish, "Bombay Duck." *Bacterium phosphoreum* and *Sarcina littoralis* predominated. They have also investigated the epidemiological aspects [47] by measuring the survival of *E. coli*, *S. typhosa* and *Vibrio comma* in the tissues of the fish. They find that these organisms in the tissues could survive normal cooking process.

Iyer and Bhat [48] have determined the thermal resistance of *B. subtilis* in distilled water, neutral phosphate and industrial brine.

Saxena *et al* [49] have developed a simple medium for the growth of *Vibrio cholerae*.

Harikishin [50] has developed a method to reutilize MacConkey's broth for *B. coli* presumptive test.

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ENZYMES

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Conforming to established convention, this literature survey comprises the researches carried out and published in India, as well as by Indians abroad, in the field of enzymology during the calendar year 1953. These investigations have covered a wide range of enzymes. While most communications pertain to well known systems, a few authors have obtained evidence of the presence of new ones. Some of the contributions are primarily concerned with the nature, mode of action, and co-factors of certain important enzyme systems. The literature has been dealt with under the following heads: (A) Non-oxidative Enzymes, (B) Oxidative Enzymes (C) Miscellaneous types, (D) Inhibitors and Activators, and (E) Other Aspects.

(A) Non-oxidative Enzymes

Rao, Birnbaum and Greenstein [1] found that hog kidney acylase-I hydrolyses N-acylated-L-amino acids (except glycyl-L-isovaline), but not the corresponding D-amino acids. Dehydropeptides (excepting N-acylated dehydroalanine), all compounds with alkyl substituents in place of the α-hydrogen atom of the terminal amino acid residue, amino acid amides and chloracetyl dipeptides are not hydrolysed. N-glycyl peptides, not containing any D-amino acids in the chain are hydrolysed at an appreciable rate. Based on the non-susceptibility of β alanyl-L-histidine to the enzyme the authors draw the conclusion that acylase-I is a different carnosinase which also occurs in hog kidney homogenates.

Chandrasekhara and Swaminathan [2] found that ragi in the ungerminated state has very little amylase activity which develops markedly on germination. The α-amylase content of ragi malt, in contrast to barley malt, is higher than the β-amylase content, Both these enzymes contribute equally to the saccharifying action of ragi malt on starch. The saccharogenic activity of ragi malt is slightly lower than that of barley malt. Optimal conditions for the

satisfactory yield of malt are found to be 72-96 hours of germination at temperatures between 23° to 28°C, after preliminary steeping for 24 hours. Similar findings were reported by Saroja and Giri.[3] In contradistinction to the optimum pH of 7 for α-amylase in the resting grains of barley and ragi, the optimum pH for the same in the germinated grains was found to be 4.5-5.0.

In a review article on amylases and their industrial applications Lulla [4] has classified naturally occurring amylases indicating the sources rich in them and has discussed the technological and economic advantages of the enzymes derived from microorganisms over those from other natural sources.

Saxena [5] detected arginase activity in the hepatopancreatic gland of the terrestrial snail, *Achatina fulica* (Fer). No arginase was present in those specimens of *A. fulica* which had hibernated for six months.

Agarwala, Mohan Rao and Shrivastava [6] adduced chromatographic evidence for the presence of a γ-peptidase, which hydrolyses glutathione, in the cells of *Vibrio cholerae* (*Ogawa-52*) The cysteine liberated, upto a concentration of 0.03% in the culture medium, is completely metabolised. Addition of cysteine to the culture medium does not hinder the hydrolysis of glutathione, which suggests that glutathione hydrolysis is not primarily for the supply of cystine to the organism.

Doss and Saraswat [7] reported a decrease of 20-25% in the activity of invertase in presence of 6.7% boric acid. In a study of the kinetics of inactivation of invertase at 25°C in acid media the same authors [8] obtained indications of the existence of two processes, one very fast at comparatively high pH values, and the other at low pH.

Saraswat [9] has shown that detergents decrease the activity of invertase, cationic detergents being more effective than the anionic types. In presence of the latter class, the critical pH of inactivation is found to shift to higher pH values.

Lal and Kanugo [10] found invertase activity in the livers of *Palamnaeus bengalensis*, the common large Indian scorpion.

The lipolytic activity of seven varieties of castor beans has been reported by Nazimuddin and Kulkarni.[11]

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Lulla and Johar [12] found that among the molds they examined *Penicillium notatum* had the highest pectinase activity. Maximum pectinase activity was obtained from *Penicillum notatum* grown on powdered lucerne as the substrate.

Radha [13] observed a lowering in the pectin content of French bean plants infected with *Macrophomina phaseoli* (Maubl.) Ashby. That this is due to the presence of pectolytic enzymes present in the pathogen has been sought to be proved by *in vitro* studies on cut bits of potato with enzymic extracts of the fungus.

Experimenting with mycelial felt of *A. niger*, Krishnan and Bajaj [14] obtained enough evidence to establish the distinctive nature of metaphosphatase, although they have not been able to effect a complete separation of metaphosphatase activity from apyrase and pyrophosphatase.

Working with spores and mycelium of *A. niger*, they have further shown [15] that the polyphosphatase activity varies significantly during growth, sporulation and autolysis, and bears a definite relationship to the concentration of phosphoric esters. The authors take this as indication that the polyphosphatases here are not mere structural units, but along with other enzymes play a definite role in the metabolism of the mold.

Sundararajan and Sarma [16] have demonstrated the presence of phosphoprotein phosphatases in the spleen, liver and kidney of the adult rat, rabbit, cattle, sheep and pig. Among these rat spleen has the maximum activity. The enzyme (a purified preparation from ox spleen, for example) activated by thioglycollic acid, acted specifically on only phosphoprotein (casein), and had very little action on the proteolytic breakdown products or on glycerophosphate.

Chandrasekhara and Swaminathan [17] extracted with acetate buffer of pH 5, 85-90% of the pyrophosphatase activity of ragi malt powder. The optimum pH for the activity of the enzyme was 5.0 and temperature near about 45°. The dialysed enzyme extract was slightly activated by Zn^{++} (5×10^{-4}M) and was highly inhibited by fluoride (1×10^{-3}M); Mg^{++} was without effect. Unlike proteases and amylases in ragi, even the resting grain had some pyrophosphatase activity.

In studying the effect of germination on the phosphorus content (phytin-and water-soluble inorganic P) of eight species of common Indian pulses, Belavadi and Banerjee [18] reported that phytase activity, which is absent in the ungerminated pulse, appears in the germinated pulse and that phosphatase activity is also increased by seed germination.

Using gelatin as substrate and viscosity as the index of activity, Agarwala, Shrivastava and Pant [19] found that the optimum pH of the protease in *Vibrio cholerae* is around 8. Incorporation of gelatin into the growth medium considerably enhanced the enzyme activity. Reducing agents increased the activity in some strains but NaCN usually showed inhibition. Cu^{++} inhibition could be partially reversed by glutathione, suggestive of SH groups in the enzyme molecule. Antibiotics activated the enzyme from the Inaba and Ogawa strains, but had no effect in water tank strains.

Chandrasekhara and Swaminathan [20] showed that the protease activity in ragi which is low or negligible increases considerably on germination. The optimum pH is 4.4 and temperature 37°C. The enzyme is activated by H_2S, HCN and inactivated by Cu^{++}.

Continuing their previous work on the milk-clotting enzyme of fig latex, Krishnamurti and Subrahmanyan [21] have further purified the enzyme. Regarding the question, whether the very many catalytic functions this preparation possesses are performed by a single entity or by different enzymes, their results show the latex preparation to be a mixture of enzymes. For this study, the authors have invoked the aid of methods of adsorption and fractional precipitation with alcohol. Thus the fraction precipitated by 66.7% alcohol gave the maximum milk-clotting activity. This was most sensitive of all the catalytic functions to treatment with adsorbents. Norit charcoal, Kaolin and Fuller's earth at a pH range of 4 to 5 adsorbed 100, 95 and 75% respectively of the milk-clotting activity, which was destroyed by about 60% by adsorption on charcoal.

Streblus asper, a shrub which grows wild in the semi-arid regions of South India, contains a milk-clotting enzyme distributed

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in all parts of the plant. Confining their studies to water-extracts of the leaves of the plant as the source of the enzyme, Sri Ram and Reddy [22] have used acetone for fractionating the enzyme. They find that the activity is impaired during dialysis and ultra-filtration and attribute it to oxidation. They find no linear relation between enzyme concentration and its activity. Boiled milk is clotted faster than raw milk.

Bhimeswar and Sreenivasaya [23] described a method for the preparation of ribonuclease from castor seeds. The preparation, which is free from proteolytic enzymes and desoxyribonuclease, was found to have pH and temperature optima of 5 and 60°C respectively.

Arora, Dudani and Krishnamurti [24] studied the aryl sulphatase activity of several pathogenic bacteria using potassium 2-hydroxy - 5-nitrophenyl-sulphate as substrate. Glucose, fructose and galactose inhibted the enzyme markedly, maltose and lactose to a less extent, while sucrose, raffinose and starch had no effect at all.

(B) Oxidative Enzymes

Williams and Sreenivasan [25] extracted choline dehydrogenase from rat liver mitrochondria with sodium choleate and phosphate buffer as the dispersing system. The fact that the soluble enzyme utilises 2, 6-dichlorophenol indophenol but not oxygen as hydrogen acceptor indicates that not all the mitochondrial components of the choline oxidase system are extracted by choleate. The extraction procedure gave a 13-fold purification of the enzyme. Among other enzymes tested mitochondrial succinic and betaine aldehyde dehydrogenases were also brought into solution by choleate.

Williams and Sreenivasan [26–29] also investigated in detail the tyrosine oxidase system of rat liver with particular reference to the co-factors involved. The following findings have been recorded; (1) The total tyrosine oxidase system is completely soluble in 0.25 M sucrose. (2) At high levels, 2, 6-dichlorophenol indophenol (DCPP) markedly inhibits the enzyme through destroying ascorbic acid, thus confirming that ascorbic acid is an essential co-factor of the system. (3) Another co-factor is glutathione. (4) A unique phenomenon concerning tyrosine oxidase is the marked stimulatory

effect of DCPP at low concentration. (5) Ascorbic acid functions at two points in the sequence of reactions in tyrosine oxidation; one early in the scheme in the oxidation of phenylpyruvic acid, and the other later, in the conversion of homogentisic acid to acetoacetate and fumarate. (6) In addition to glutathione and ascorbic acid a labile factor, for which a low concentration of DCPP can substitute, is another co-factor. (7) Ageing and dialysis result in rapid inactivation of the enzyme (8) Activity can be fully restored to the dialysed enzyme by the combined addition of ascorbic acid, glutathione and an extract of heated fresh enzyme. The enzyme extract contains, in addition to pyridoxal phosphate, which is essential for the transamination of tyrosine, a factor necessary for the maximal oxidation of tyrosine. This factor and the DCPP-substituting factor are possibly identical. Based on the evidence presented, the authors have proposed a tentative scheme for the enzymatic oxidation of tyrosine indicating the sites of action of the various co-factors.

Dudani *et al.*[30] demonstrated dehydrogenase activity in the Ogawa strain (No. 49514-Kasauli) of *V. cholarae* as well as the Inaba strain and the rough variants derived from it. Formate, fumarate, pyruvate, lactate and succinate, various amino acids, and glucose all function as hydrogen donors.

(C) Miscellaneous Types

An acetate activating enzyme, which catalyses the reversible conversion of acetate and coenzyme A to acetyl coenzyme A and simultaneously of adenosine triphosphate to adenosine monophosphate and pyrophosphate, was prepared by Ramakrishnan and his collaborators[31] from pig and rabbit heart. The interaction of ATP with CoA to form AMP and pyrophosphate proceeds in the absence of added hydroxylamine provided calcium is present. The preparation contains AcCoA and deacylase. The concentrations of CoA and glutathione (or cysteine) Mg^{++}, ATP, acetate and hydroxylamine control the rate of reaction. The reaction itself is reversible. The enzyme is active only with acetate and propionate and has not been resolved so far.

Iyer *et al.*[32] showed that resting cells of *V. cholerae* deaminate

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aspartic acid over a pH range 6.5 to 9. Deamination was most active at pH 8 and found to be a bimolecular reaction, aerobic in nature and of the oxidative type.

Having shown by circular paper chromatography that γ-amino butyric acid is present in the acid hydrolysate of red yeast, *L. glutinis* var. *rutescens*, Krishnaswamy and Giri [33] carried out experiments to prove the logical conclusion that this amino acid in red yeast is formed, as in other sources, by the enzymic decarboxylation of glutamic acid.

Padmasini Ayengar and associates [34] found that an aqueous homogenate of mycelial pads from *A. fumigatus* catalysed the formation of glutamic acid from α-ketoglutarate and γ-aminobutyric acid or β-alanine, when the mold was grown on ammonium chloride and glucose. The transaminase activity was increased 3 to 7 fold when the sole nitrogen source of the medium was either γ-aminobutyric acid or β-alanine. The fact that these preparations can transaminate glutamic acid with phenyl pyruvic, α-ketoisovaleric and pyruvic acids, while γ-aminobutyric acid only showed activity with α-ketoglutarate suggests that transamination may be the chief reaction by which the nitrogen of γ-aminobutyric acid is rendered available for the mold. Acetone powders of glutamate—or acetate—grown *E. coli* formed glutamic acid readily from γ-aminobutyric acid and α-ketoglutarate, but not from β-alanine. Preparations from *Lactobacillus arabinosus* had no activity with either amino acid. The aminobutyric transaminase was obtained in solution from acetone powders of acetate-grown *E. coli* and some purification was achieved.

Mitbander and Sreenivasan [35] adduced evidence for the presence of a thermolabile (enzymic) principle in intact rat red blood cells which can convert pteroyl glutamic acid to the citrovorum factor. This conversion is considerably diminished if the incubated mixture contains added aminopterin.

(D) Inhibitors and Activators

Studying enzyme inhibition in relation to drug action, Ghatak and Krishnamurti [36] observed that the alkaline phosphatase activity of a crude extract of rat kidney, as measured on sodium

β-glycerophosphate in veronal buffer of pH 6-8 as substrate, is inhibited by dihydrostreptomycin and terramycin. Mg^{++} affords protection against this inhibition. Penicillin G and chloromycetin exert a slight activating effect. Aureomycin prevents the inactivation of the enzyme by sodium cyanide. Terramycin inhibition is of the competitive type. Iyer, Arora and Krishnamurti [37] reported that antibiotics inhibit rat liver arginase which is reversed by Mn^{++} or Co^{++}.

Vijayaraghavan and Narasinga Rao [38] traced the low nutritive value of duck's egg white, which improves remarkably on autoclaving, to the existence of an antitryptic factor in the ovomucoid. In the presence of the ovomucoid, the release of histidine, lysine, threonine and valine from casein by trypsin is highly inhibited, while the liberation of isoleucine, leucine and tryptophane is moderately inhibited and that of arginine, methionine, and phenylalanine is not significantly retarded.

Nath and Hatwalne [39] recorded that the succinic oxidase activity of rat liver homogenates is inhibited to the extent of 79.2% by acetoacetate in a concentration of 0.04M. The inhibitory effect is considerably reduced by incubation of the acetoacetate with the homogenate prior to addition to the substrate. Reduced glutathione (GSH) protects the enzyme if it is present before the addition of the inhibitor, but does not reactivate the inhibited enzyme.

Braganca and Quastel [40] studied the inhibitory effect of snake venoms—Russel viper venom, cobra venom and the venom from *Agkistrodon piscivorus*—on a number of oxidative enzymes. Small quantities of the venoms heated for 15 minutes at 100°C inhibited the enzyme systems concerned with the oxidation of glucose, fructose, pyruvate, L-glutamate, succinate and α-ketoglutarate in brain homogenates. Pyruvic dehydrogenase of brain, succinic dehydrogenase of brain and heart, cytochrome oxidase of brain and choline oxidase of liver were particularly vigorously inhibited. Anaerobic glycolysis in rat brain, glucose fermentation by yeast, pyruvic acid dismutation by pigeon liver, hexokinase, glucose oxidase of liver and of fungi (notatin), soluble pyruvic dehydrogenase of pigeon breast muscle, lactic and malic dehydrogenases, urease and in general all enzymes active in cell-free

extracts, were not affected. It is suggested that the heated venoms contain a thermostable enzyme, probably a lecithinase, which attacks the phospholipins or lipoproteins of the mitochondrial structures upon whose intactness and configuration depends the activity of the several enzyme systems. That the inhibitory effect of the venoms is enhanced by calcium ions which activate lecithinase lends further support to this view.

Braganca, Faulkner and Quastel [41] observed that in rat brain slices the inhibitory effect of ammonium ions on the synthesis of acetyl choline is considerably diminished by methionine sulfoxide, ethionine sulfoxide and methionine sulfoxime, substances which inhibit glutamine synthesis. L-glutamate and DL-methionine had no such effect. Extra ammonium ions in respiring brain slices increase the rate of formation of glutamine, thus reducing the available ATP and hence the rate of synthesis of acetylcholine.

Braganca, Quastel and Schucher [42] found that DL—methyl glutamate (0.01M) inhibits glutaminase activity in brain tissue by 73%, being comparable in this respect to D- and L-glutamate. Under conditions favouring the synthesis of glutamine, ammonia absorption by DL—methyl glutamate also occurs with the formation of L-methyl glutamine.

Basu and Whitaker [43] found that the cellulase from *Myrothecium verrucaria* was inhibited to varying extents by salts of heavy metals, by oxidising and reducing agents, iodoacetate, and p-chloromercuribenzoate (I). Inhibition by heavy metals and I was reversed by SH—compounds or other reducing agents. It is suggested that thiol groups are essential for the enzyme action. At low pH levels, acid dyes were inhibitory, inhibition increasing with decreasing pH ; at higher pH levels, basic dyes were inhibitory. In the lower pH range, basic dyes and neutral salts, such as those of proteins, were stimulatory.

Datta and Basu [44] reported that quinine (in a concentration of 10^{-3}M) inhibits the oxygen uptake by enzymes of the tricarboxylic acid cycle prepared from the liver and kidney of the white mouse, excepting when succinate is used as the substrate.

(E) Other Aspects

Shanmugasundaram and Sarma [45] observed *in vitro* synthesis

of nicotinic acid from D-tryptophane by rat liver but not by cattle and rabbit liver slices. They ascribe this to the presence or absence of D-amino acid oxidase in the respective tissues. Similarly, germinating green gram has been shown to be devoid of D-amino acid oxidase activity and is unable to synthesise nicotinic acid from D-tryptophane. These findings were further confirmed by Sundaram and Sarma [46] with rice-moth larvae which lacked D-amino acid oxidase activity as well as ability to utilize D-tryptophane for nicotinic acid synthesis.

Williams, Sreenivasan, Sung and Elvehjem [47] found that folic acid deficiency in rats, induced either by a diet containing sulfasuxidine or by the administration of aminopterin, resulted in low choline oxidase activity of liver mitochondria. Microbiological tests revealed that under these conditions the folinic acid content of the liver was reduced, but the folic acid content was unaffected. Based on these findings, the authors suggest that folinic acid is concerned in the maintenance of normal choline oxidase activity in liver mitochondria.

In collaboration with Williams *et al.*, Sreenivasan [48] observed a lowering in the endogenous respiration and in the xanthine oxidase and transmethylase activities of liver homogenates from rats rendered B_{12}-deficient. Choline oxidase activity was slightly increased. These changes in the liver enzyme systems were not greater when a B_{12}-antagonist, prepared by the oxidation of the vitamin with hydrogen peroxide in strongly acid solution, was administered to the B_{12}-deficient animals.

Mathur [49] observed no correlation between polyphenol oxidase content and weight of potato tubers, but there was a parallelism between oxidase content and respiratory activity. The polyphenol oxidase was prepared by Szent György's method and its activity determined manometrically in terms of oxygen uptake.

Bhat, Broker and Iyer [50] report that the aqueous extracts of saffron stimulate the activity of digestive enzymes, while nutmeg exerts a somewhat opposite effect on all but the gastric enzymes. The enzymes subjected to these tests were salivary amylase, pepsin, trypsin, rennin, pancreatic amylase and pancreatic lipase.

Vaidyanathan and Giri [51] demonstrated the presence of arginase, in both the bound and free forms, in the germinated seedlings of the Field bean (*Dolichos lablab*). Heavy metals such as Mn ++, Co++ & Fe ++, markedly activated the enzyme system. Prolonged dialysis or acetone precipitation of aqueous extracts of the enzyme removed one or more essential co-factors.

Jagannathan and Singh [52] found all the component enzymes of the Embden-Meyerhof-Parnas glycolytic system (hexokinase, phosphohexoisomerase, phosphofructokinase, aldolase, phosphoglyceraldehyde dehydrogenase, phosphoglyceromutase, enolase, phosphopyruvic kinase, carboxylase and alcohol dehydrogenase) in mycelia of high citric acid yielding strain of *Aspergillus niger*. The authors described a simple method for obtaining cell-free extracts of these enzymes and procedures for proximate assay of their individual activities.

Ganguly and Deuel (Jr.) [53] confirmed the presence of vitamin A esterase activity in rat liver homogenates and traced its location to the microsomal fraction.

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VITAMINS

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Contributions made during the year under review relate mostly to vitamin A and its provitamins. Several interesting studies on niacin and vitamin B_{12} have also been reported.

Provitamins and Vitamin A

Datta and Rajagopal [1] investigated the excretion of vitamin A in different pathological conditions. They observed no parallelism between excretion in urine of vitamin A, of proteins and of purines and the white blood cell count. Most of these urines, from pathological cases, however, took up measurable amounts of vitamin A into solution. Freshly voided normal urine was also found to take up measurable amounts of vitamin A, when shaken with non-saponifiable fraction of halibut liver oil. They also studied the effect of addition of plasma-protein fractions of Cohn and extra amounts of purine bodies to urine on their dissolving capacity of vitamin A. Kolah and Radhakrishna Rao [2] investigated the comparative functions of vitamin A and essential fatty acids and the effects of their deficiencies on the skin of rats. They observed that the degree of hyperkeratinization and plugging were inversely proportional to the amount of vitamin A in the diet. The essential fatty acid deficiency produced cutaneous lesion very similar to that produced by vitamin A deficiency. Differences however were observed in the manner of deposition of Keratin material and the lining epithelium of the hair follicle and sebaceous gland in the two deficiencies. The authors conclude that deficiencies of vitamin A in conjunction with essential fatty acids play an important rôle in the production of cutaneous lesion in rats resembling those in human phrynoderma, and suggest that phrynoderma may be due to combined deficiencies of both these factors. Sadhu and Ghosh [3] studied the influence of vitamin A on the tissue redox system and in sulphate metabolism in guinea pigs. From their observations, the authors postulate that the biological function of vitamin A in

the body which affects the distribution of it in sulphate, is primarily due to its effect on the tissue redox systems.

Ganguly and Krinsky [4] observed that a direct relationship between the plasma and the liver vitamin A alcohol could not be confirmed in as much as despite, wide variation in the concentrations of vitamin A alcohol in the liver, the plasma level remained relatively constant. The relative distribution of vitamin A ester and vitamin A alcohol in the various cell fractions of rat liver homogenates was studied by Krinsky and Ganguly [5] by the technique of differential centrifugation. Vitamin A ester was found to be concentrated in a centripetally migrating material and the alcohol form was present in the centripetally migrating material as well as in the supernatant and microsomal fractions. The mitochondrial and nuclear fractions were essentially free of vitamin A. Esh and Bhattacharya [6] carried out studies on the absorption and utilisation of vitamin A palmitate by feeding it to vitamin A deficient rats, both massive and small doses, dispersed in arachis oil, ethyl oleate or in aqueous medium with the help of dispersing agents. With massive dosage, highest liver storage was obtained from the aqueous medium. With small dosage, various diluents seemed to have no significant influence on the growth rate or storage. The effect of fortification with anti-oxidants was also studied. Chanda [7] carried out an investigation on eight lactating cows of the effect of mastitis on the carotenoids, vitamin A and phosphorus compounds of milk. He observed an increase in the total carotenoid content but the percentage of β carotene remained unchanged. The total vitamin A content of the milk fat remained unchanged but the percentage of vitamin A present in the alcohol form was increased. Kamath and Magar [8] estimated vitamin A, both by spectrophotometric and glycerol dichlorhydrin methods, in 10 samples of liver oil from 6 different species of fish. The value ranged from 1,000 to 32,900 I.U./g.

Biswas, Dakshinamurti and Das [9] determined the carotene content of twenty-three fresh green leafy vegetables. All the green leaves studied were found to contain chiefly the β form of carotene. Zechmeister and Karmakar [10] detected the presence of very small quantities of phytofluene in chlorophyll containing tissues of 21

species representing 10 families of vascular plants.

Bhatia [11] determined the vitamin A content of cows butter fat in the Punjab. The value ranged between 17.5 and 61.0 I.U./g. The vitamin A content of the butter fat decreased with the progress of lactation.

Ganguly, Mehl and Deuel [12] have demonstrated that carotenoids present in different tissues of chicken were always of adventitious origin. When the birds were maintained on a carotenoid-free diet over a prolonged period, the tissues were completely free from carotenoids. Moreover, in separate experiments only the carotenoids administered to such birds appeared in various tissues. Hydroxy carotenoids appeared in large amounts while hydrocarbon carotenoids appeared only in traces.

Ganguly *et al.*[13] have found that the variation in absorption and deposition of carotenoids in tissues of animals is dependent upon the species. They have discussed at length the question of variation in tissue distribution of different carotenoids in different species of animals and have finally suggested that this is due to appropriate protein receptors present in the tissues.

Goodwin, Malini Jamikorn and Willmer [14] carried out studies on carotenogenesis in phycomyces and observed the action of diphenylamine in inhibiting the synthesis of β carotene and in increasing that of phytofluene group. No compound of similar structure or oxidation reduction potential has been found which has the same effect on carotenogenesis as diphenylamine. Ferrous sulphate does not counteract the action of diphenylamine. Riboflavin at lower concentration stimulates carotenogenesis and at higher concentration inhibits the process.

Thiamine

Venkateswara Rao [15] describes a deficiency syndrome in children occurring in Hyderabad (Deccan), characterised by diarrhoea, slight fever, and generalised oedema. The cases recovered with vitamin B_1 plus liver extract. Balakrishnan and Rajagopalan [16] studied intestinal thiamine synthesis in rats with three different types of fats—butter fat, cocoanut oil and groundnut oil. Butter fat produced the best results. A preponderance of coliform

bacteria in the faeces and caecum of the butter fat fed rats than the other two groups was also observed. In further work they observed that with 13 gm. of curds accounting for only 5 γ of thiamine, the urinary and faecal excretion of thiamine is considerably more than from the corresponding animals receiving 10 γ of synthetic vitamin.[17] The reduction in thiamine excretion when sulfaguanidine is fed is much higher in the synthetic thiamine group than in the curd group Probably curd-feeding results in developing sulfa-drug antagonism similar to that when p-aminobenzoic acid is administered. The authors point out that this observation has a fundamental and practical importance in nutrition more specially in the case of persons who generally take curds or butter-milk with every meal. Giri [18] describes a simple and rapid method which can be used for the simultaneous separation, identification and quantitative determination of thiamine and riboflavin by circular paper chromatographic technique. Moorjani and Bhatia [19] describe the methods for the processing of groundnut milk, and report only its average chemical composition. The method is also described for the preparation of curd out of this milk. Figures for thiamine, riboflavin and nicotinic acid for these products are given. Joshi, Mater and Magar [20] have determined the thiamine, riboflavin and nicotinic acid content of 24 varieties of Bombay fish.

Nicotinic Acid

Shanmugasundaram and Sarma [21] have shown that while rat liver can synthesize nicotinic acid from D-tryptophane, cattle and rabbit liver cannot. The utilisation of the D-isomer is ascribed to the presence of D-amino acid oxidase in rat liver and to its absence in cattle and rabbit livers Chattopadhya *et al.*[22] studied the metabolism of nicotinic acid in rabbits and observed that these animals do not excrete trigonelline as a metabolite of nicotinic acid but excrete a negligible amount of NMN. The authors have also suggested modifications in the method of estimating trigonelline, nicotinuric acid and NMN. Shanmugasundaram, Tirunarayanan and Sarma [23] demonstrate the influence of biotin on the formation of nicotinic acid from tryptophane. Prasad, Gupta, Biswas and Das [24] have compared three chemical methods of estimation based

upon the well-known Konigs' reaction and compared them with the microbiological method. They have found Chaudhuris' method using CNBr and p-amino-benzoic acid to be simple and accurate giving results comparable with the microbiological method. Chaudhuri [25] describes a method for the estimation of nicotinic acid in normal urine as well as in urine containing a high percentage of glucose. Reddi and Kodicek [26] studied the excretion of metabolites by man and rat after administration of nicotinic acid, nicotinamide and tryptophane by paper chromatographic techniques. After a dose of 100 mgm of nicotinic acid to man, the main nicotinyl metabolites in the urine were, in decreasing order, nicotinuric acid, N methyl-nicotinamide and nicotinamide. Nicotinic acid appeared in the urine only when the flushing of the skin and other vaso-dilatory symptoms occurred.

In the rat, nicotinic acid appeared consistently in the urine. The administration of nicotinamide to human subjects caused an increased excretion of N-methyl-nicotinamide. Rat urine contained, in addition, increased amount of nicotinic acid. Nicotinuric acid was not excreted by either of the species. After ingestion of tryptophane, human urine showed an increase in the excretion of N-methyl nicotinamide, while rat urine had in addition an increased output of nicotinic acid.

Choline and Folic acid

Ahmad, Karim and De [27] report the choline content of 27 varieties of fishes and 33 common foodstuffs of vegetable origin on moisture—free basis and conclude that fishes, leafy and root vegetables are excellent sources. Choline dehydrogenase has been extracted by Sreenivasan and Williams [28] from rat liver mitochondria with sodium cholate and phosphate buffer as the dispersing system. Sreenivasan *et al.*[29] studied the relationship of the deposition of folic acid and folinic acid to choline oxidase of isolated rat liver mitochondria.

Biotin

Tirunarayanan and Sarma [30] investigated the influence of biotin on the gammexane-inositol relationship in neurospora crassa

and observed that biotin effectively prevented the inhibition of growth by gammexane, inosital is only partially able to overcome the growth inhibition by gammexane The authors suggest that biotin may be concerned with the synthesis and utilisation of inositol in the organism.

Vitamin B_{12}

Van Klaveren, Banerjee, Srivastava and Patel [31] describe a rapid method of photometric analysis of Vitamin B_{12} in crude liver extracts.

Sreenivasan *et al.*[32] studied the effects of vitamin B_{12} deficiency on liver enzymes in the rat. The authors have discussed these observations with respect to other known facts concerning vitamin B_{12} metabolism. Das gupta, Chatterjee and Basu [33] treated 21 cases of macrocytic anaemia in pregnancy with vitamin B_{12} by oral and / or parenteral route and observed that the response by the parenteral route was better than by the oral route. The authors suggest that an average case of macrocytic anaemia in pregnancy represents combined deficiency of folic acid and Vitamin B_{12}.

B complex

Radhakrishnamurthy and Sarma [34] separated and identified most of the B group vitamins by ascending paper chromatography. Sivaramakrishnan and Sarma [35] studied the influence of para amino benzoic acid, sulfanilamide and folic acid on amino acid changes during germination.

Manna and Hauge [36] found that vitamin B_{13} (the rat growth factor from distiller's dried solubles) had some physical and biological relationship with orotic acid. On rats, 'distiller's soluble factor' (vitamin B_{13}) has got slightly higher growth response than orotic acid. Due to the low biological potency of orotic acid compared with that of vitamin B_{13} and the slight difference in their absorption spectra, it was concluded that orotic acid might be a decomposition product of vitamin B_{13}.

Ascorbic Acid

Gangadharam and Sirsi[37] investigated the role of ascorbic acid in experimental tuberculosis. They are of the opinion that ascorbic

acid has a definite retarding effect on the progress of the disease in guinea pigs as indicated by the survival time, gross amount of lesion, etc. Chakravarty, Mondal Das and Chaudhuri [38] are of the opinion that contrary to the observation of Chatterji, ascorbic acid and antihistaminics do not appear to have any effect in the prevention and treatment of ureamia in cholera.

Nath, Saha and Chitale [39] have studied the role of acetoacetate in aggravating the disturbances in the carbohydrate metabolism of scorbutic guinea pigs. Rudra [40] has adduced further evidence of the intermediary role of manganese in the biosynthesis of ascorbic acid in rats and rabbits. Signs resembling scurvy in guinea pigs have been produced in rats and rabbits by putting them on a synthetic diet complete in other known essential nutrients but lacking in manganese.

Sidappa and Bhatia [41] have investigated the role of pH in the xylene extraction method for the estimation of ascorbic acid. Dhopeshwarkar and Magar [42] observed that ascorbic acid in canned guava was compietely available to the body as synthetic ascorbic acid. Siddappa [43] carried out the estimation of ascorbic acid in Indian hog plum and observed that the concentration of "apparent ascorbic acid" is 4-5 times that of true ascorbic acid in the plum which is approximately 6-10 mgm 100 Gm.

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HUMAN NUTRITION

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During the year under review the subjects of immediate importance in India, such as nutritional oedema, fatty degeneration of the liver, fluorosis, etc. have continued to receive attention at the hands of investigators.

Clinical, physiological and field studies

Mukherjee and Werner [1] have noted that in cases of nutritional oedema 30% of the total intake of fat in the diet was excreted unabsorbed as compared with 4% in the case of normals. With improvement in their clinical condition and the reversal of the albumin globulin ratio the absorption of fat improved and approached that of the normals. From studies of liver biopsy specimens in 30 cases of *kwarshiorkar* of children under 10 years of age, Sriramachari and Ramalingaswami [2] conclude that treatment with skim milk powder could reduce the fat and cholesterol that had accumulated in the liver. Prolonged fatty infiltration of the liver, according to them, resulted in diffuse hepatic fibrosis. Chaudhuri [3] has discussed the pathogenesis, pathology, symptomatology and management of nutritional disorders in children suffering from oedema.

Chakravarti *et al.*[4] have studied 12 cases of epidemic dropsy and found that in these cases, the total protein of blood serum was usually within normal limits but albumin was reduced and globulin raised. As the clinical condition improved with treatment, the available thiocyanate space showed a decrease from 331.5 ± 46.0 ml. to 247.3 ± 39.3 ml. per kg. of body weight and the interstitial fluid from 244.0 ±40.7 ml. to 163.2 ±32 5 ml. per kg. of body weight.

Govindan and Radhakrishna Rao [5] report that environmental temperature plays a role in the dietary production of fatty liver in albino rats. They found that on the same diet of 10% casein and 30% lard, the liver fat value was 0.52 g. per 100 g. of body weight

in winter (November-December), while in summer (April-May) when the mean temperature was higher by 10°F it was 1.04 g. per 100 g. body weight. Patwardhan *et al.*[6] have studied the progressive changes in livers of rats when given a single injection of CCl_4 at the end of varying periods of time interval between 6 and 120 hours. There was a reduction in the liver succinic dehydrogenase and transaminase, and an increase in alkaline and acid phosphatase and glutamic dehydrogenase. They also found that even though total nitrogen in the liver was reduced, methionine content remained unaffected.

Govil *et al.*[7] carried out a diet and nutritional survey of 412 boys living in 14 districts of Uttar Pradesh. The diets were found deficient in proteins of animal origin, calcium, vitamin A and ascorbic acid ; these dietary defects were reflected in the health of the boys. In a survey of a food scarcity area of Bihar, Lal [8] found that the people there suffered from malnutrition, loss of body weight and oedema of the legs with a significant lowering of the total blood protein levels. From an investigation carried out in a village near Madras city of an outbreak of night blindness, Someswara Rao *et al.*[9] observed that among the aetiological factors responsible for causing the condition may be calorie under-nutrition and hook-worm infestation, these exerting some effect on the metabolism of vitamin A.

Nutritive value of foodstuffs

Venkataraman and Chari [10] reported that the edible portion of the Indian chank (Xancus pyrum) had a high percentage of protein similar to that of fish and therefore advocated their consumption on a large scale.

Proteins and fats

In an attempt to find out the alleged toxicity of Indian pulses, Bengal gram (*Cicer arietinum*) and green gram (*Phascolus radiatus*), De [11] kept young rats on a diet of 33 parts pulse, 66 parts rice and 1 part sodium chloride. The rats developed paresis of the hind legs followed by loss in body weight and death. He concluded that these effects were due to a deficiency of calcium

and phosphorus in the diet and not due to the presence of any toxic material in the pulses.

The Council of Scientific and Industrial Research has published the results of investigations carried out in 5 different laboratories in India on the nutritive value of vanaspati as tested on albino rats and humans.[12] It was found that the comparative nutritive values were in the following descending order: first ghee, second raw groundnut oil, refined groundnut oil and vanaspati of m. p. 37°C., and third vanaspati of m.p. 41°C.

Mineral metabolism

In experiments with rats, Sathe and Krishnamurthy [13] observed that the iron in autoclaved soya bean was better absorbed by the animal if any one of the following organic acids, *viz.*, tartaric, citric or ascorbic (vitamin C), were fed at about 60 mg. per rat per day along with the soya bean. In further experiments with rats, they observed that the absorption of iron supplied as ferric citrate in the diet was inversely proportional to the amount of phytin phosphorus in the diet.[14] In the above experiments, the phytin phosphorus supplied was in the form of any one of either unpolished, 7% polished, or 15% polished, raw rice.

Venkateswarlu and Narayana Rao[15] analysed 173 samples of water for fluorine in the Vishakapatam area of the Madras State and found them to contain from a trace to 11 p.p.m. They have suggested a house-hold method for the removal of fluorine from water used for drinking purposes; the method depends on the property of rapid absorption of fluorine by magnesium oxide added to the water while it is boiling.[16]

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ANIMAL NUTRITION

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The published reports on research in animal nutrition during the last two years under review (1952 and 1953) were fairly extensive and varied. Semi-scientific articles on the various problems connected with animal feeding were also numerous.

Kehar [1] has discussed in great detail the problem of animal nutrition and its bearing on human welfare to prove that the relation between the cattle population and human beings is one of inter-dependence and not of competition. In the course of this discussion he has suggested ways and means of bridging the gap in the huge shortages of cattle feed and human food. He has also pleaded for better appreciation of the work in the laboratory, and for the closer association between the laboratory and the field activities. Two symposia were held on (*a*) the problem of food deficit in India [2] and (*b*) the physiological efficiency of man and animal as conditioned by climatology and nutrition.[3]

Dev Goswami [4] has discussed the food problem of Assam *vis-a-vis* the number and condition of its cattte population. According to him the solution lies in the elimination of the State's surplus useless cattle, growing of more fodder crops, utilization of the indigenous fodder grasses, and preservation of the glut of monsoon grasses for scarcity periods. He also pleads for the provision of more and balanced ration to the useful and productive section of the cattle population. Basu [5] has given a review of the role of trace elements in the nutrition of animals and has warned against the indiscriminate use of supplements of trace element mixture as a routine procedure, as this might be harmful at times.

In the field of research on subsidiary feeds, Kehar and his colleagues have continued their sustained efforts on the utilization of waste materials as cattle feed. Kehar [6] has described how mango seed kernel, normally a waste, which is a rich source of

carbohydrates and protein, can be successfully utilized as a cattle feed. When crushed and mixed with oil cakes or grains the animals develop a taste for it and it can form upto 20-25% of the total concentrate mixture. The kernel has been fed with advantage to all classes of cattle and buffaloes, and the animals maintain bloom and health even on long term feeding. Experiments on rats showed that mango seed kernel flour can be incorporated in wheat or any other *atta* upto 5% for human consumption. The kernel, however, needs a little processing for this purpose. The use of mango-seed kernel and jaman-seed meal, first advocated by Kehar and associates, has been extended by Bose and co-workers [7] in poultry feeding. They have reported that inclusion of these waste materials upto 20% of yellow maize meal in a normal growing ration showed no detrimental effect. Continuing their studies, Kehar and Negi [8] reported that mangrove leaves (*Avicennia officinalis Linn*) are a good roughage in the feeding of cattle. The trees are found to thrive well even in salt marshes, and in Kutch, when famine makes all other fodders scarce, this is available in plenty. Mangrove leaves have been found to meet almost half the total roughage requirement of a ration, and to supply almost as much digestible crude protein and total digestible nutrients as would be available from Pipal (Ficus religiosa) leaves. The palatability of the former is, however, comparatively low, but when it is intimately mixed with wheat bhoosa the animals relish it. Patel and Srivastava [9] have found that feeding of tobacco oilcake has no adverse effect on the animals. Dastur, Ray and Sen [10] have discussed the use of fodder yeast and urea in the ration of cattle. Yeast, they state, is rich in protein and low in fibre, and contains fat and minerals in fair amounts. Dharmani and Karira [11] have investigated the usefulness of maize cake as cattle feed. They state that although maize cake is rich in fat content (19.8%), no untoward effects whatsoever were noticed on the general health of animals. They recommend its feeding in conjunction with green berseem so as to get the best results.

Grassland research has been the subject of a very large number of publications during the past two years. Dabadghao,

Sen and Bains [12] have studied the effect of different fertilizers on the yield and chemical composition of Rhodes grass (*Chloris gayana*) and Lucerne (*Medicago sativa*) mixtures. The application of phosphate fertilizers, in the form of superphosphate alone or in combination with ammonium sulphate, and as ammonium phosphate, has been found to improve significantly the yield and the quality of the fodder. Sen and Bains [13] have continued their studies on the effect of manuring on the quantity and quality of fodders. They have shown that the application of superphosphate improves the quality of the fodders in regard to phosphate and lime but without any effect on the nitrogen content. Dabadghao and Gandhi [14] have also reported on the chemical composition of 13 important grasses and legumes at different stages of maturity. The nutritive value of the grass locally known in Assam as Nalini (*Themeda quadrivalvis* O.KTZ) has been studied by Das, Mukherjee and Sen.[15] This is a highly draught resisting species that grows luxuriantly, and has been found very palatable even at the flowering stage. Fed alone, it maintains positive balances of the nutrients studied except calcium, which is slightly negative. Das and Mukerjee [16] have also reported on the nutritive value of joy-joha (*Ischaemum rugosum* Salisb), another indigenous grass of Assam, as a cattle feed. They observe that even when fed alone, the grass can maintain positive balances of calcium and phosphorus, and in this respect it stands unique among most of the indigenous grasses found in Assam. At the flowering stage, the grass contains 7.13% crude protein. The digestibility coefficient of the grass, both as green and hay, is fairly high. The hay by itself provides a maintenance ration with regard to protein. Malik [17] describes the varieties of fodder crops which have been evolved or introduced with marked degree of success in the Punjab States.

Kehar and Sahai [18] have shown that when animal feeds are arranged in the ascending order of their copper content, the cereal straws constitute the first group, green feeds the second group and concentrates the third. On the basis of manganese content, however, the grains and straws rank lowest and are followed by concentrates and green feeds forming the second group and brans the third.

When arranged in the ascending order of the iron content, the grains and oil seeds come at the top followed by cereal by-products, dry roughages, green feeds, oil cakes, and animal by-products in respective sequence. Copper, cobalt, manganese and iron contents of feeds like other minerals, have been found to be a function of the age of the plant and to vary with soil and other environmental conditions in which the feed is raised. The same authors have also examined several organs and tissues of the body for the trace elements. They find the copper content highest in liver, followed in order by lymph gland, bone-marrow, pancreas and spleen. In regard to manganese, liver is followed in order by pancreas, kidney, bone-marrow, testes and brain. Cobalt and manganese contents of blood and tissues have not generally been found to vary with season. Metabolic investigations on ruminants show that a ration consisting of wheat straw and rape cake although capable of registering positive balances for major nutrients like nitrogen, calcium and phosphorus, give a negative balance for copper. Similarly, a ration consisting of wheat straw and maize cake is found deficient in manganese; another of barley and oat hay, in iron. The average minimum copper, cobalt, manganese and iron requirements of ruminants worked out on the basis of metabolic studies have also been reported.

Narindar Nath and Das [19] have shown that monthly cutting maintains the protein, phosphoric acid, lime and ether extract contents of different grasses at fairly high level. There is a close parallelism in the variations of crude protein and phosphate, while lime generally follows a trend reverse to both. At the end of the investigation they, however, observed, a sharp decline in all the values. Narindar Nath and Das [20] have continued their studies on the effect of stages of growth on the chemical composition of some more grasses; of these *Iseilma laxum* has been found rich in protein, ether extract and mineral contents. Paul [21] has given the chemical composition of the Australian Blue grass (*Paspalum dilatatum*), which is now the most commonly grown pasture grass of Assam and appraised to be highly nutritious not only as a green forage but also as hay or silage. Lakke Gowda [22] has reported the results of a study on the chemical composition of jowar

(*Andropogon sorghum*) as affected by stages of maturity. The results show that as the plant reaches full bloom there is a progressive increase in organic matter, crude fibre, and nitrogen free extract, and decrease in crude protein, total ash, calcium, phosphorus and magnesium. Gupta [23] describes the use of cowpea as green fodder for cattle in *Kharif* season (as berseem is in the *Rabi*). Pointing out its importance in increasing milk production, he suggests its extended use for silage and hay making; the latter to be used during the scarcity periods. Srinivasan,[24] while giving details of the method of cultivation of sorghum and maize, states that as both of them are low in protein for milk production, mixed cropping of these with legumes would be desirable.

The results of a rather extensive balance experiment to study the effect of manuring on the nutritive value of some fodders, the influence of the latter upon milk yield, its composition and mineral retention in 4 high yielding milch cows of the Sahiwal breed, have been reported by Mathur and Desai.[25] The fodders studied are unmanured berseem, manured berseem, green lucerne and jowar silage *plus* lucerne hay with supplements of chalk and salt. It has been observed that during the 7 weeks of experimental feeding no significant change takes place in live weight and milk yields nor in the mineral composition of the milk due to the alteration in the feeding schedule. The manured berseem is found to lead to the highest positive calcium balances. This is followed by unmanured berseem and lucerne. Positive balances are, however, maintained in all cows under the different regimes of fodder feeding. On the other hand, phosphate balances, except in the case of manured berseem, have been negative in all other cases. The results of the investigation further show that 100-200 gm. CaO/day in the feed can meet the requirement for 30-40 lbs. of milk yield. The same amount of P_2O_5, however, is inadequate for the purpose. The authors have also noticed that assimilation of mineral nutrients is better from the green fodder than from hay or silage.

Feeding of colostral fat for calf raising has been the subject of a communication by Sen and co-workers.[26] The results of feeding colostral fat, at 3% level in skim milk, to calves from the age of 6 days upto about 3 months indicate that skim milk reinforced with

colostral fat is a good substitute for whole milk in the raising of young calves. The results of a growth study with Sindhi and Gir heifers on the effect of supplementing the ration with iodinated casein have been reported by Sen *et al.*[27] They have found that during the 28-week feeding period when iodinated casein is supplemented in the ration at the rate of 2 gm/100 lb. live weight the growth of the heifer is retarded. This retardation in growth takes place inspite of the fact that animals receiving the iodinated casein exhibited the same capacity for digestion of food. From the metabolism data they have also concluded that the ingested nutrients under the induced hyperthyroidism have been used up for purposes other than growth. Mullick, Murty and Kehar [28] have reported the effect of implantation of 20 mg. of stilbestrol and of feeding 1 gm. of thiouracil daily with the concentrate, on the metabolic turnover of the proximate principles and minerals. The average dry matter consumption and T.D.N. between the control and stilbestrol groups, they find, do not show any significant variation. In the case of the thiouracil group, however, the animals show lower ingestion of dry matter and T.D.N. The balance of nitrogen, calcium and phosphorus and the water metabolism are not significantly affected by the different treatments. Records of electro-cardiogram in experimental animals show slower rate of heart beat; potentials in Q.R.S. and T waves are, on the other hand, greater as compared to the control group.

Talapatra [29] has discussed the biochemistry and technology of silage making, especially of the type best suited to Indian conditions. Gupta [30] gives the chemical composition of the silage made out of a combination of whole sugar-cane and green oats. This silage, he states, compares very favourably with that of jowar silage in nutritive value. Kehar [31] writing on the subject of improving straws by alkali, water or lime treatment, gives details of the various methods of treatment for improving their feed values. He describes also the feeding experiments conducted on calves and milch cows, etc. with the treated straws at different centres, where actual feeding trials were conducted, and the benefits derived therefrom. Summing up, he states that paddy straw which suffers from certain inherent disadvantages can be improved by treatment with

dilute alkali solution, especially in those places where water is available in abundance and at least 100 animals are maintained. Individual livestock owners too, he points out, could improve such straws by simple soaking in water and washing. Again, in places where water is in scarcity, this wash water can be further utilized by adding 0.5% lime in the latter. These latter two substitute treatments, however, are stated to be less efficient as methods of improving the straw quality.

Climate *vis-a-vis* animal health and feed utilization have been the subject matter of several publications. Thus, Mullick and Kehar [32] have recorded observations at monthly intervals on six Kumaon steers on the physiological reactions of their body and inferred that the changes in air temperature appeared to be the major cause affecting variations in these reactions and that the respiration rate is one of the indices for assaying heat tolerance of animals. Mullick, Murty and Kehar [33] in another communication point out that the amount of water taken and the insensible perspiration are the highest in summer and lowest in winter, and the dry matter intake varies with water intake. They further state that the ingestion of protein and fat is much lower in summer and autumn than in winter and spring. Similarly, the amount of digested protein is less in summer and autumn months whereas that of digested carbohydrate is higher during the same period. The same authors [34] conclude from metabolism data collected all round the year that the requirement of lime is higher in summer and those of nitrogen and phosphate slightly low in winter. They state that the dietary demand of the animals to climatic stress coupled with the negative balances for nitrogen and lime in summer would necessarily warrant the need for providing roughage of better nutritional value, like hay or silage, instead of cereal straws. Seasonal variation in heat production of cattle and buffaloes has been the subject matter of another communication by Mullick and Kehar.[35] They observe that the heat production per hour of Hariana, cross-bred cows and Murrah buffaloes decrease with the increase in air temperature.

Murty and Kehar [36] have recorded the normal values of morphological and chemical constituents of blood of Kumaoni bullocks.

Chopra *et al.*[37] describe a rapid method of determining fat in oil seeds. The method involves sampling, grinding, extraction with benzene from a mortar, filtration and evaporation. They claim that the method is economical and is comparable to the conventional methods in regard to accuracy of result. Biswas and Das [38] describe a method of chromatographic separation of carotene in materials containing lycopene. The method makes use of a specially prepared mixture (50 : 50) of magnesium carbonate and kieselguhr. Rudra and Rudra [39] describe a micro-method of estimating selenium, which involves the use of ascorbic acid instead of hydroxylamine for the reduction of selenious and selenic acids. It is claimed that by this method 1 μg/cc. of selenium can be estimated. Ramana Rao [40] in a communication stresses the importance of pH in the determination of molybdenum.

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PHARMACOLOGY

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A fundamental approach to the study of problems pertaining to the mode of action of drugs and investigation of many Indian medicinal plants have been the activity at many pharmacological laboratories. Considering the innumerable problems awaiting immediate attention in pharmacognosy, pharmaceutical chemistry and chemotherapy of, the many reputed, clinically useful indigenous drugs the pace of research is rather inadequate.

The publication of pharmacognosy of Ayurvedic drugs by the Central Research Institute, University of Travancore, has given to the hands of the research workers a source of reference of the pharmacognosy of some plants[1]. This is the first volume of a series expected to fulfil this essential need. From an account of the attempts made to cultivate some medicinal plants in Kashmir, it is observed that plants now being cultivated on a commercial scale include pyrethrum, *D. purpurea*, *D. lanata* and *Atropa belladonna*. The results of experimental cultivation of many other species are mentioned. A plea is made that more attention should be paid to indigenous drug industry and experimental cultivation should go hand in hand with chemical analysis.[2] The Council of Scientific and Industrial Research's endeavour in bringing out the Indian Pharmaceutical Codex [3] is also timely. A compilation of this type is a guidance to clinical teachers and paves the way for an Indian pharmacopaea pertaining to indigenous drugs, to be used as a standard by the Indian Pharmaceutical Industry.

Malaria

No new discovery has been made during the year either in the field of drugs or the clinical status of the existing antimalarials. Attempts to synthesise more potent and less toxic antimalarials are being carried on. Compounds of guanidine series, biguanides,

triguanides and bis guanidines have been prepared and screened for antimalarial activity in chicks.[4] None was found superior to paludrine although a few compounds exhibited significant and comparative antimalarial activity. Synthesis and testing of some N^1-sulphanamide phenyl N^5-isopropyl biguanides and N_1-Aryl sulphonyl N^5-aryl biguanides [5] have shown only moderate activity with the former and no activity with the latter series. Tests of substituted thiocarbomide derivatives of metalinamide, metachloridine and sulphanilamide, on blood induced *P. gallinaceum* infection in chicks have shown metalinamides to be slightly active and the rest to be inactive.[6] Of the twenty-four N^1-N^5 substituted biguanides synthesised and screened in chick malaria none was found effective.[7] A few of the synthetic antimalarials of the sulpha guanidine derivatives screened against *P. gallinaceum* have been found to possess marked suppressive activity.[8]

Chemotherapy of malaria with special reference to the work done in India [9] is an interesting review of the work done by some of the laboratories in India. Comparative efficacy of quinine, paludrine, chloroquin as regards clearance of trophozoites from the peripheral blood has shown the combined treatment of paludrine and mepacrine to be most efficacious.[10] An analysis of 62 human malaria cases shows that Daraprim is slower in action than chloroquin or Camoquin and it failed to cure 10 out of 20 cases of falciparum malaria.[11]

The use of antimalarials in other protozoal and bacterial infections has also been considered. Camoquin [12] in the treatment of Giardiasis has proved successful, all but one of the twenty-eight cases being rendered free of this flagellate. N^1-N^3-Bis (Aryl and Allyl thiocarbanyl) metanilamides and substituted thio urea derivatives of metanilamide [13] have been investigated, for the *in vitro* antibacterial and antitubercular activity.[14]

Micro-electrophoretic study of serum proteins from normal and malarial chicken (infected with P. gallinaceum) has been conducted and the ascending and decending interferometric electrophoresis patterns described.[15]

The following were the observations in a study of the action of antimalarial drugs on enzyme systems.[16]

Quinine inhibits the O_2 uptake of the enzymes of the tricarboxylic acid cycle except when succinate is used as the substrate; the inhibition is partially reversed by BAL and H_2S water; quinine inhibits the formation of succinate from α keto-glutarate in competition with arsenite and quinine stimulates the enzyme activity at the succinate to fumarate step.

Tuberculosis

Myco-tuberculosis with its wide powers of adaptation is still not completely amenable to chemotherapuetic agents. The testing of certain antimalarials as tuberculostatic agents has already been referred to.[13,14] Along with the synthetic drugs the attention is being directed to plant products for the treatment of tuberculosis. The oil from Leeahirta [17] is found to inhibit the growth of myco-tuberculosis as also of staphylo-aureus and past-pestes in concentrations of 10 μ, 100 μ and 50 μ per ml. respectively. Sesamin,[8] a crystalline material obtained from oleum sesame while exhibiting little antibacterial activity against common pathogens inhibits the growth of tubercle bacilli in a fairly high dilution. In experimental tuberculosis of guinea pigs [19] it has been shown that vitamin C has a definite retarding effect on the progress of the disease and the tuberculin reaction becomes positive later in vitamin C fed infected animals while vitamin C depletion renders the reaction positive earlier.

Indian medicinal plants

In spite of the spectacular development of the synthetic compounds, vegetable drugs still have and will continue to occupy a prominent place in the physicians armamentarium.

Special mention should be made of *Rauwolfia serpentina* which has been in use in indigenous medicine for a long time and of late has suddenly come into prominence for treatment of high blood pressure. The adrenergic blocking activity of purified total alkaloids and of the oleo resinous fraction of *Rauwolfia serpentina* (Benth) [20] on various effector organs is found to resemble closely the known sympatholytic drugs with a competitive adrenaline antagonism. Using the total purified alkaloids of *Rauwolfia*

Serpentina in monkeys further evidence is produced in detail of the central origin of the hypotensive and vasomotor blocking actions of these alkaloids.[21] Rauwolscine,[22] an alkaloid obtained from the leaves of another species, *Rauwolfia canescens* Linn, is also found to possess hypotensive action and to depress the tone and peristalsis of intestines. It is optically active, forms colourless plates of M. P. 231-232°C and has the chemical composition $C_{21}H_{26}O_3N_2$. The hypotensive action of serpasil or preparation 13020 another alkaloid from *Rauwolfia serpentina*, on clinical trials [23] is found to lower the systolic and diastolic blood pressure in majority of cases with no serious side reactions whatsoever.

Chaksine, an alkaloid from *Cassia albus* Linn, is found to possess weak curariform action [24] and produces a fall in blood pressure chiefly by causing capillary dilatation. Its M. L. D. for rats is approximately 70 mg/kg. Chaksine sulphate inhibits the growth of staph. aureus in a dilution of 1/10,000.[25]

A new cardiac active aglycone corchogenin has been separated from the seeds of *Corchorus olitorius* Linn.[26] The bioassay of the genin shows its digitalis like cardiac action.

Isolation and identification of steroid sapogenins from the yams of 19 different species of dioscorea plants grown in different parts of India have been undertaken to find out a rich Indian source for diosgenin or any other steroid sapogenin suitable as a starting material for the preparation of cortisone.[27]

The biochemical changes involved in the action of some plant extracts on the pathogenic organism have shown that *Ocimum sanctum* seeds, *Ocimum basilicum* and *Magnifera Indica* seed kernels possess the property of markedly delaying the coagulase producing capacity of pathogenic staphylococci.[28] Besides the direct action on the organism, plant extracts also exert their action through the host. *Murraya koenigii* leaf extracts raise considerably the phagocytic index and cause a mobilisation of the leucocytes to take part in phagocytosis.[29] Though possessing no *in vitro* activity on staphylococci, the leafy juices of spinach, betel, kukronda, neem and cabbage are shown to activate the healing process in experimental wounds and ulcers,[30] best effect being obtained by local application of juice of kukronda leaves.

The effects produced by pure sanguinarine hydrochloride and alkaloid free argemone oil show that these substances do not produce manifestations of human epidemic dropsy in Rhesus monkeys.[31] The toxic effects and the changes in the vascular pattern in argemone fed animals indicate that 1% or more is toxic to the tissues while 0.5% may also be toxic after prolonged use.[32]

Endocrinology

The intra-muscular injections of progesterone, serum gonadotropin and testosterone propionate show that these cause atrophic changes and decline in ascorbic acid content of rats adrenal cortex,[33] the androgen recipients showing the maximum injury.

The influence of melanophor hormone, shown to be free from A.C.T.H. upon the synthesis of melanin pigments in the skin of the frogs by the increased formation or synthesis of melanin in localized areas has disproved the claim that ACTH and the melanophore hormone are identical.[34]

The results obtained from the action of acetylcholine and adrenaline on isolated segments of intestine of kitten suggest that the acetylcholine effect seen at the end of three minutes with adrenaline is due to the formation of ACTH, in the intestinal wall and the compound responsible for its formation is adrenochrome derived from the oxidation of adrenalin.[35]

Industrial toxicology

The nature of lesions caused by asbestos and mica in experimental pnuemonokoniosis in guinea pigs reveals that in the case of lung mica dust predisposes to nodular formation whereas asbestos dust causes only diffuse fibrosis.[36]

Miscellaneous

Fluorine toxicosis is a health hazard in certain endemic areas. The metabolism of fluorine,[37] pathological changes in the tissues of rats and monkeys in fluorine poisoning,[38] and the studies on endemic fluorosis have been conducted by various workers. An advanced manifestation of fluorosis involving selected impairment is found to be associated with consumption of water containing 10 p.p.m. of fluorine.[39]

A study of the effect of temperature on the liberation of acetylcholine confirms that for a given activity of the vagus an increase in temperature to 35°C. is accompanied by a directly proportional increase in the liberation of Ach in frog's heart. Above 40°C. the amount liberated decreases.[40]

Turpentine is a better solvent than kerosene oil for extraction of alkaloids from nux vomica seeds and is more economical since it can be recovered by steam distillation and reused.[41]

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PLANT PHYSIOLOGY

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During the year under review, the Plant Physiological investigations, carried out in the country, dealt mainly with problems of mineral nutrition, of development and of plant hormones and movements.

Nutrition

Lal and Rao [1] studied the effect of 21 ratios of N, P and K upon growth of barley grown under soil culture in pots. On reproductive growth, an increasing N/P ratio was found to be favourable, while high P/K ratios were not so promising. On vegetative growth, increase in N/K ratio appeared favourable. N/P, P/K and N/K ratios of 6 : 1, 4 : 1 and 3 : 1 appeared on the whole best for grain yield.

Dastur and Kanwar Singh [2] studied the effect of applications to the soil of molybdenum, boron, copper, zinc, iron, manganese, chromium and magnesium on the growth and yield of the American upland cotton (Indore I), under rain-fed conditions, at Indore. The experiment was started in 1947-48; in the succeeding two seasons (1948-40 and 1949-50) half the quantity of each element, used in 1947-48, was added to the respective plots and in the following two seasons, these applications were entirely omitted. The object of this mode of application was to see the residual effect, if any, of these applications. The data of yields indicated an overall beneficial effect of copper, boron, zinc, manganese and chromium.

Govindan [3] described symptoms due to deficiency and excess of zinc in tomato.

Singh and Singh [4] investigated the causes of die-back in Mandarin and sweet orange at Saharanpur. The symptoms were: bark excrescences, gum pockets, stained terminal branches, multiple buds and die-back They eliminated fungal attack, lack of drainage, hard pan, soil salinity, irrigation water, etc., as the contributing factors and inferred that deficiency of copper may be the cause of this disorder.

Ganguly [5] reviewed the results of investigations on the shedding of buttons in the coconut.

Development

Sircar and Sen [6] studied the development of the shoot apex in rice, variety Rupsail, under different photoperiods. The initiation of the reproductive phase in the stem apex was marked by a preliminary elongation, followed by a depression at the tip and by development along the edges of small groups of specialized cells which protruded and ultimately developed into branch rachises bearing more than one spikelet. A minimum exposure for 4 weeks to short day (8 hours) was required for 100% flowering of the main shoot, whereas with exposures for 3 and 2 weeks only 66 and 3% of shoots flowered respectively.

Singh [7] reported that shoots from tubers of *Cyperus rotundus* Linn entered the reproductive phase about 30 days earlier than the control, after a low temperature treatment (7°C) lasting for four weeks.

Misro, Misra and Sastri [8] from a study of the photoperiodic behaviour of certain varieties of rice, suggested that " the fact that there is no response to photoperiod treatment with seedlings of a particular age should not lead one to believe that the type under consideration is photoperiodically insensitive. The treatment should be tried at various ages of the seedlings before any such conclusions can be drawn."

Shukla [9] found that on chilling seeds of *Lens esculenta* at 6-9°C for 32 days the resulting plants flowered earlier by about 32 days. Exposure was quantitative in its effect on flowering in that the effect of the treatment was exerted only on the primary axis and the tiller buds present, the tillers initiated later not flowering early.

Misra [10] found that on exposure to short day (8 to 10 hours until ear emergence) variety D. 1.4 of rice flowered after about 87 days, whereas the control flowered after about 7 months. The grain yield was, however, considerably reduced (as compared to controls) due to reduction in spikelet and grain number under the high temperature prevalent after ear emergence.

Hormones and Plant movements

Samantrai, Misra and Kabi [11] found that petioles of detached leaves of *Dolichos lablab* Linn., *Ipomoea batatas* Lam and *Canavalia ensiformis* rooted very well as a result of treatment with I.A.A., N.A.A., and I.B.A. High concentrations of the first two (100 and 50 P.P.M.) were required for the desired effect in contrast with the low concentration of I.B.A. (2 5 P.P.M.).

Samantrai and Kabi [12] induced rooting in petioles of the leaves of *Amaranthus gangeticus* Linn. var. tristis by treatment with 5 P.P.M., beta indolylbutyric acid and transplanted the isolated leaves with such roots. After a month the petioles became thicker and stouter due to anomalous secondary growth peculiar to the stem of the species. The authors regard this observation as an evidence in support of Arber's partial shoot theory of the leaf.

Dikshit [13] succeeded in inducing rooting in cuttings of Early Round, Howe and Kelsey by treatment with I.B.A.

Misra and Samantrai [14] induced roots on the petioles of isolated leaves of *Dolichos lablab*, *Canavalia ensiformis*, *Lantana camara*, *Congea tomantosa*, *Heliotropium indicum*, *Tropaeolum majus* and Malvaviscu sp. by treatment with very dilute aqueous solutions of beta indolylbutyric acid and alpha naphthylacetic acid.

Choudhari and Gaur [15] investigated the effect of some amino acids and growth-regulating substances on the survival and rooting in cuttings of *Duranta plumieri*. Unsoaked cuttings could not stand adverse weather conditions and most of them died off. Soaking in water (for 15 hours), previous to planting, initiated quicker rooting and increased the survival. Treatment with 10 P.P.M., indolepropionic acid gave the longest roots and the highest survival percentage. The glutamic acid (10 P. P. M.) treatment was characterized by the formation of prolific foliage and high survival.

Moolani [16] found an accelerated development of seedlings of gram (*Cicer arietinum* L.) from seeds soaked in 2, 4-D solutions (10^{-9} and 10^{-8} M.) for 20 hours.

Choudhari and Bhatnagar [17] found that bolting in onion was suppressed by application of maleic hydrazide as a spray (0.25%) and to the soil (0.025%). The size and quality of the bulbs were

also improved as a result of these treatments.

Bose [18] proposed a physico-chemical theory of tropic, diurnal and irritability responses in plants. As regards tropic curvature in growing coleoptiles, he suggested that indoleacetic acid (I.A.A.) is formed by the action of an enzyme on tryptophane, the production, however, not being continuous. The I. A. A. so produced, diffused to regions of lower concentration. The I.A.A. is continuously being inactivated by an enzyme EA, this reaction being hindered by a side reaction between EA and an inhibiter M.

As regards the mechanism of transmission of excitation in sensitive plants, Bose assumes that an irritability substance (IS) when released or activated by the passage of a current of excitation causes a local depolarization of the plasma membrane, resulting in a flow of electric current to it from intact regions. Thus the excitation travels along the conducting channel by a series of local currents till it reaches the pulvinus.

As regards the diurnal movement of the leaves, Bose bases his theory on Bunning's idea about the autonomous changes in the acidity of the plant sap which sets in when the plant passes from the photophile to the skatophile state. Bose assumes that oxidase responsible for the oxidation of IS is inactivated by light. The resulting increase in the concentration of IS in the pulvinus during the dark period will, therefore, bring about contraction.

Dutt [19] gives an account of the tissue modifications of organs associated with transmission of stimulation and with mechanical response in *Mimosa pudica* Linn. The features in the pulvinus which would appear to facilitate bending are : transeversely furrowed epidermis, absence of collenchyma and aerenchyma, adaxial cortex being thicker walled and smaller celled than the abaxial absence of pith, location of phloem in the centre, presence mostly of spiral elements in the xylem and absence of any specialized or 'nerve' cells.

Dutt, Guha Thakurta and Dutt [20] have studied the autonomous pulsatory movement of the leaflet of *Oxalis repens* Thunb. The young leaflet completes about six pulses during the day, whereas at night the movement stops, the leaflet undergoing a sleep movement. The mature leaflet pulsates throughout day and night,

the freqnency during the night being comparatively less. With the supply of 1% glucose solution the young leaflet pulsates throughout day and night and the sleep movement at night establishes itself if the supply of sugar is withheld.

Acid metabolism

Seshagiri and Sastri [21] found that the titratable acidity of the leaves of *Tamarindus indica* Linn increased with age, showing a tendency to fall slightly in the oldest leaves. No diurnal fluctuation in the acidity of leaves of different ages was found. The titratable acidity was the highest in the winter and the lowest in summer. The leaf sap had a high buffering capacity.

Bharucha and Shankar [22] found that in a non-succulent plant like *Amaranthus spinosus* with high pH there were certain features of Crassulacean metabolism such as, acidification due to low temperature and the opposite effect by a high temperature; however no diurnal fluctuation in acidity was observed.

Miscellaneous

Lal and Rao [23] have suggested a formula for estimating plant dry weight from (*i*) the length and width of leaves, (*ii*) leaf dry weight/leaf area ratio, (*iii*) leaf number and (*iv*) leaf dry weight/plant dry weight ratio.

Guha Thakurta and Dutt [24] studied the diurnal variations as well as variations during the continuous growth process of cinchona, sunflower and dahlia. In general, most of the growth occurred at night and during the day the growth was very low or negative. Pronounced rhythmical pulses were observed in the diurnal growth of sunflower and dahlia, the rhythmicity being less pronounced in cinchona. Diurnal growth in diameter also followed a similar course. The results are accounted for on the basis of J. C. Bose's theory regarding rhythmicity in plant growth.

Dutt and Guha Tahkurta [25] investigated the effects of different chemical and gas treatments on shortening the dormancy of freshly harvested tubers of potato varieties, Nainital and Darjeeling Red Round. Treatments with 2% solutions of sodium, potassium and ammonium thiocyanate and 1% ethlene chlorhydrin shortened the

dormancy of half-cut tubers by 5-6 weeks. Similarly vapour treatment with ethylene chlorhydrin (0.5 c.c./l) was effective on half-cut tubers. It was also observed that the resting period of immature tubers was shorter than that of the mature ones. The incapability of the less dormant immature tuber to germinate in the field, so long as it is in contact with the mature plant, was ascribed to the secretion of some inhibiting hormone from the mother plant. The effect of CO_2 in breaking dormancy of the tuber was attributed to a direct stimulatory action of the gas and not to anaerobiosis indirectly brought by absence of oxygen.

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SOILS AND FERTILIZERS

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In the wake of Post-war reconstruction schemes to ameliorate the rural conditions of the country, more particularly in relation to the river valley projects, attention is being focussed on soil erosion as a major problem affecting land economy. The magnitude of this problem was set out by Chaturvedi [1] and measures for relief delineated. The Central Government has set up a Central Board of Soil Conservation with a view to combating soil erosion and increasing the fertility of soils. A chain of soil erosion research stations have been proposed and some of these were started during the year to study the problems region-wise and to educate the people with regard to the scientific methods of attacking the problems. Work on these and other problems were extensively studied during the year under report.

Soil Surveys, land utilization and classification : Mukherjee[2] discussed the necessity for the preparation of a soil map and also a map of the Basic Soil Regions of India in relation to their practical value for agriculture and utility for plant introduction. These maps which represent various soil types, climatic conditions and special features in soil conditions will serve as useful guides to soil scientists and research workers.

Agarwal [3] discussed the programme of soil survey work in progress in Uttar Pradesh which includes: (1) a reconnaissance survey intended to locate the major soil regions found in each district, and (2) detailed soil survey involving intensive study of soil variations in selected 'teshils.' He has given data to link up the crop response to major fertilizer ingredients like nitrogen and phosphorus with the soil types found in different parts of the State.

Detailed physical and chemical studies of soils from two profiles from Delhi and three profiles from Ajmer have been studied and reported by Tamhane, Shome and Raychaudhuri.[4] From the profile features it is suggested that the Delhi and Ajmer soils may be grouped under grey soils. In certain respects, like xerophytic

vegetation and semi-arid climates, the soils of Delhi appear to be like the Prairie soils of the United States which border the Eastern edge of the meridianal belt of Chernozen soils in the United States.

Nath [5] discussed the problems of land utilization in India and Dutt [6] outlined the need for planned land utilization and proper soil management in the areas under the Damodar Catchment.

The soils of the Vindhya Plateau in the Mirzapur District of Uttar Pradesh are described by Agarwal, Mukerjee and Verma [7] and three types, *viz.*, the red, the brown and the black are distinguished, as occupying three topographic positions in the Catena. From the data of the composition of the clay fractions from the three soil types a weak laterization was recognised, while the soils were not considered as typical laterites or even lateritic.

Subrahmanyam [8] described the survey of Hindupur taluk in the Ceded Districts with special reference to waste land areas. Govindarajan [9] reported the survey of the soils of the Vanivilasa Sagara Tract in Mysore State and classified the soils according to their suitability for wet (Paddy) and garden cultivation. The soils of the inner Himalayas were described by Sen [10] who found them rich in nitrogen and phosphate. As the interior of the Himalayas was approached progressively the soils became more and more shallow except in depressions, more acid, more rocky, contained less lime, but more phosphates and sometimes more potash. The narrow C/N ratio of the soils were interpreted to suggest easy nitrifiability of the soil organic matter indicating good fertility. A review of the pre-irrigation soil surveys done in different parts of the country was give by Dewan [11] and the procedure and methodology adopted in the pre-irrigation survey in the Damodar Valley detailed.

Soil erosion and its control : The measures for checking soil erosion through conservation methods which have been adopted in Madras State were detailed by Venkatachari [12] and Reddy [13] and suggestions were made for dealing with the problems of soil erosion in : (a) State forests and pasture lands and waste lands, and (b) private holdings cultivated or left waste. Tamhane [14] discussed the need for regional approach to land utilization and management with the object of furthering soil conservation methods. The

importance of soil conservation in multipurpose river Valley Projects in India was stressed by Bannerjee.[15] The problem of silting and measures for its reduction were discussed by him and his experimental results were found to confirm the previously established formula relating sedimentation to erosion.

Physical and physico-chemical properties of soil: Roy and Das [16] studied the electro-chemical properties of hydrogen clays from several Indian soils in relation to their mineralogical make up. Hydrogen clays were prepared from horizon samples of four typical soil profiles. Electrometric titration of these clays with baryta and variation in viscosity of their aqueous suspensions with increasing addition of sodium hydroxide have been shown to give valuable information for identification of their mineralogical make up. The hydrogen clays from Padegaon black cotton soil have strong monobasic acid character, suggesting their montmorillonitic nature. This was confirmed by their electroviscous properties, chemical composition and thermal dehydration studies. From similar studies the mineralogical character of the clays was inferred for a yellow *matasi* soil, Delhi soil and Karnal soils.

Dyal [17] using different identification methods showed that the clay fraction of Gainsville loamy fine sand of Florida consists of 60 to 80% of leptyls of mica and 4 to 7% of Wavellite. The surface areas and cation exchange capacities of seven other soils containing predominantly Kaolin or Kaolin-gibbsite mixtures in the clay fraction were lower than those of the Gainsville soil. He has suggested that the high degree of zinc fixation observed in this area may be due to the high exchange capacity of the mica leptyls or the Wavellite content of the Gainsville soil.

Marshall and Patnaik [18] in a review of the literature on the physico-chemical properties of soil humic matter pointed out that cations were held on the whole less firmly by humus fractions than by clay minerals. The cationic bonding energies changed little near the inflection points on the pH curves. In the fraction from the peat, sodium was held more firmly than potassium and magnesium more firmly than calcium. The fractions from the chernozen showed much smaller differences.

Puri, Lakhanpal and Verma [19] brought forward a novel

theory to explain the phenomenon of plant respiration based on the spontaneous property of charcoal to form a surface layer of CO_2. By virtue of the formation of this surface layer, charcoal underwent surface ionization in water, yielding H ions which neutralised alkalies and decomposed carbonates in stoichiometric proportions. The layer of molecular CO_2 formed on the surface appeared to be 1 molecule thick. This ability of charcoal to absorb O_2 and release it as CO_2 threw fresh light on the mechanism of plant nutrition and the intimate relationship between the soil and root hairs. The transference of ions from one surface to another coupled with the evolution of CO_2 in root hairs would explain how these ions are transported as carbonates.

A mathematical equation has been developed by Banerjee and Banerjee [20] to connect the field strength at any given distance from the transmitter and the soil constants of the region, both for vertically and horizontally polarised waves. The values of the conductivity and dielectric constant of the soil were computed by comparing the theoretical curves between field strength and distance with those obtained by practical observations.

The use of clay-membrance electrodes for the determination of copper ion was described by Mitra and Chatterjee [21] who found that calcium-montmorillonite membrance prepared by heat treatment at 600°C give satisfactory results with copper solutions within a fairly wide concentration range.

Mitra and Sawhney [22] studied the reaction between montmorillonitic hydrogen clays and neutral salts with reference to the crystalline structure of montmorillonite and features of the titration curves of the hydrogen clays as acid systems. The reactions between the hydrogen clays and neutral salts were interpreted as consisting, partly, in an exchange of H ions on the surface for the cations of the salt and partly as a decomposition or disruption of the clay lattice leading to the liberation of cationic Al-complexes. The complementary ion interaction in which one cation affected the exchangeability of another in clays, was studied by Mookerjee and Mukherjee [23] who found that colloid chemical reactions involving ions of similar valencies showed in general, additive behaviour, whereas those of dissimilar valencies illustrate antagonism.

Chemistry of soil organic and mineral constituents : Bannerjee, Bray and Melsted [24] attempted to determine the extraction of cobalt employing some commonly used solvents under varying soil conditions and to establish the nature of the form that added cobalt assumed in the soil. Tracer technique with Co^{60} was used in studies with soils and clays. Cobalt added to soils was found on extraction to be present either as an easily exchangeable form which could be extracted by normal neutral ammonium acetate solution or as a strongly absorbed form which could be extracted almost completely by leaching with O.1 N. HCl after an hour's pretreatment. An empirical value for the ease of release of cobalt in simple exchange reactions in soils was determined.

Ram and Bhattacharya [25] made a study of the hydrolysable organic matter of Agra soils. Oven-dry samples of these soils were reflexed with HCl and H_2SO_4 of different strengths for 6 hours and the alcohol soluble and alcohol insoluble portions of the hydrolysates thus obtained were determined. Results indicated the presence of a nitrogen compound and a carbohydrate as well as inorganic elements including Al, Fe, Ca, Mg, PO_4 and SO_4 in the insoluble matter and residues.

In continuing the above work, Nagar, Ram and Bhattacharya [26] identified glycollic acid and galactose in some fractions of the acid hydrolysate. By elementary analysis N, S and Cl were detected and the action of various solvents on the insoluble and soluble fractions of the hydrolysates were recorded.

Subbaraman [27] developed a volumetric method for the estimation of phosphates based on the insolubility of bismuth phosphate in dilute acid solutions, based on the fact that diallyl dithio carbamido hydrazine forms a stable orange-red complex with bismuth. Titanic salts, ferric salts, fluoride, citrate and oxalate were found to interfere strongly. Interference by sulphate was eliminated by the addition of $Ba(OH)^2$. The method was found applicable to the analysis of a variety of phosphatic materials.

In continuing their work on changes of physical properties, like conductivity, pH and base exchange capacity of different U.P. soils, Bansal and Bhattacharya [28] studied the variations in the total exchangeable bases in the hydrated clays from these soils

when mixed with different forms of raw organic matter. The changes taking place during humification were explained as being due to hydrolysis and absorption.

Menon and Sankaranarayanan [29] have suggested the use of 40% alcohol to remove the soluble chlorides, carbonates and bicarbontes from black soils containing soluble salts and gypsum before estimating the exchangeable bases

Improved techniques in manufacture of fertilizers: The utilization of the phosphatic nodules from the Trichy District as fertilizers have proved difficult for a variety of reasons. Jayaraman and Krishnaswamy[30] tried to overcome these difficulties by adopting the thermal process for increasing the available phosphates. A small scale pilot plant to operate this process was described by them.

Gadre and Gupta [31] described the laboratory preparation of dicalcium phosphate from Egyptian rock phosphate and Indian phosphatic nodules with hydrochloric acid. The dicalcium phosphate contained about 40% of total P_2O_5 and is a neutral free flowing powder with good keeping qualities.

Carbon, nitrogen and mineral changes in soil: Sen [32] observed a positive correlation between the cane yield and the C/N ratio of a particular zone, with slight fluctuations, but there was no relationship between the C/N ratio and the sucrose per cent of juice.

Pathak [33] from two years observations on manured and unmanured soils found that the two maxima of NO_3 accumulation in the top soil (October and the summer season) coincided with maximum temperatures, when nitrification increased and NO_3 was rising from the subsoil. The water soluble salts were high during summer except P, which increased with the onset of the rains, probably due to hydrolysis and to CO_2 evolved by increased microbial activity.

A depression in evolution of CO_2 was observed by Joshi and Joshi [34] in soils to which mineral salts were added. This is attributed to the possible increase in concentration of soluble salts in soil water bringing about a physiological dryness for some of the microbial cells, resulting in their collapse and death. The lessening of the number of actively growing micro-organism on account of the concentration of the mineral matter in soil water immediately

after the addition of fertilizers to the soil may cause the lowered production of CO_2.

Soil microbiology : In the course of soil survey work in Cutch, some soil profiles were examined by Satyanarayana and Datta in the tertiary alluvial areas of little and great Ranns of Cutch. These highly saline soil profiles exhibit zones of gypsum deposition, accumulation of iron in streaks and bands and blue green gley horizons. These characteristics were presumed to be due to anaerobic micro-organic activity. Laboratory investigations showed that sulphate reducing organisms were present in all the profiles, but their activities were not uniform in the different horizons. Gypsum bearing horizons showed high activity in some profiles and negligible activity in others.

Gupta, Dudani and Shrivastava [36] examined the soils of Lucknow with a view to search for new antibiotics. An aerobic spore forming bacillus was isolated from a garden soil, the broth filtrate of which possesses antibacterial activity against both gram-positive and gram-negative bacteria particularly against the latter group.

Agnihotrudu[37] examined the microbiological population of soils in which representative plants of the Leguminosae, Pedalinacae, Malvacae and Gramineae were grown. In infected soil a significantly greater accumulation of fungi, bacteria and actinomycetes (in decreasing order) were found in the rhizosphere than in the control soils. The number of bacteria increased and fungi decreased in the rhizosphere upto the time of flowering. Bacterial numbers continued to increase in the rhizosphere of non-flowering plants and there was an increase in fungi and actinomycetes in the later stages of growth. The changes in fungal numbers corresponded with quantitative changes in Aspergillus and Penicillium Spp. Potential root pathogens (particularly fusarium sp.) were found on the root surface of almost all plants except sorghum, the rhizosphere of the latter yielding the highest numbers of fungi among non-legumes and highest numbers of actinomycetes, among all the plants studied.

In investigating the reasons for differences in fertility levels of different soils in a region, Joshi and Joshi [38] found that the

productivity of soil as measured by the yield of crops in the field is directly correlated with the microbial activity of the soil as determined by laboratory methods in so far as the bacterial numbers and nitrifying capacity are concerned. Gangadhar Singh and Shrikande[39] studied the influence of various levels of phosphorus in soluble and difficultly soluble forms on the fixation of nitrogen by Azotobacter sp. in same media. It was found that phosphorus in different forms stimulate nitrogen fixation upto a definite concentration beyond which nitrogen fixation is adversely affected. A difference in the phosphorus requirements of the old and new culture of mixed species of Azotobacter and Azotobacter chroococcum was observed. Pathak and Shrikande[40] studied the physiological activity of an Azotobacter culture isolated from Kanpur soil both in pure and crude culture in liquid and sand media.

The need for inoculation of legume seeds were detailed by Sanyasi Raju,[41] the various factors influencing successful inoculation of legumes stated and the benefit in terms of increased crop yields were given.

Minor elements in plant nutrition : On black cotton soils (pH 8.02 to 8.8) Dastur and Singh [42] found that cotton yields were increased by application of each of the elements B, Cu, Cr, Zn and Mn. Mg, Fe or Mo were not effective. With trace element application uptake of N was not increased; but Cu and Zn were absorbed at a higher rate from plots treated with these elements than from the control plots

Datta and Gurubasavaraj [43] found and that both B and Mo influenced nodulation significantly and increased fodder and seed yields of berseem, on a loam with a slightly acid pH. This work indicates the possibility of using B and Mo in conjunction with phosphates.

Agarawalla and Hewitt [44] studied in detail the effect of different sources of nitrogen on the Mo requirements of cauliflower plants grown in sand culture. The results obtained from a great variety of nitrogen sources supplying 15 millimoles/litre of N, except arginine which was given as 2 m. moles N, were discussed. It was found that all the nitrogen treatments chiefly in presence of NO_3 reduced yields particularly in the Mo deficient series.

The distribution of total manganese contained in forty soil profiles under different forms of available manganese were determined by Biswas [45] and the region and soil depthwise distribution of the different fractions discussed.

Merits of organic manures and inorganic fertilizers: The results of permanent fertilizer trials and tests with individual inorganic and organic nitrogenous fertilizers were discussed and tabulated.[46] These indicate that there was no detrimental effect from mineral fertilizers on the physical condition or bacterial population of the soil. In no case has cattle manure proved definitely superior to NPK, but its regular application produced as good yields as any other combination of inorganic fertilizers.

Shrikande [47] made a critical examination of the exaggerated claims on the merits of organic manures and particularly composts and pleaded for a judicious combination of organics which maintain a good tilth and of artificials which provide nutrients for increased production. Composts and organic manures being slow acting should follow artificials rather than be regarded as competitors of them.

Viswanath [48] quoted experimental evidence obtained in previous works to show the high value of organic manures particularly in the dry tropics and sub tropics. Basu and Rege [49] indicated that mineral fertilizers may be used indirectly to promote humus formation as *e.g.*, through fertilizing legumes or use on leys.

Bhowmick and Raychaudhury [50] discussed the available evidence to show the desirability of properly balanced fertilizers with a combination of organic and mineral fertilizers. Mineral fertilizers were a more satisfactory source of N than manure in short term trials and organics tended to have greater residual effect. In long term experiments, mixtures of mineral N and bonemeal at about 16 lbs. / acre N produced higher yields of rice than did cattle manure at 68 lbs. / acre, but the reverse was true with wheat.

The importance of earthworm activity in humus production was stressed by Fowler who brought evidence on the depressing effects of ammonium sulphate on earthworms. The effect of treatment of soils with organic manures and ammonium sulphate on the nitrification were studied by Pathak.[52] A regular loss of

carbon in all the treatments was noticed which was highest with compost and farm manure treatments.

Water requirements of crops: Singh [53] devised an apparatus for the automatic recording of losses of water from potted plants etc., the working of the apparatus being based on an electrometrical devise by which balls are released from time to time, disturbing a lever of a horizontal weighing machine whenever there is a loss in weight of the object placed on the platform of the latter.

The contribution of rain to crop yields in irrigated cultivation was assessed by Rege [54] from investigation in the Deccan Canal Tract of Bombay from pot experiments covering a period of 12 years.

Datta and Bose [55] studied the influence of depth of furrows on the irrigation of potatoes and found that under Delhi conditions 6 inch deep furrows in combination with 21" of water and 80 lbs. per acre N gave the optimum yield.

Green Manures: The effects of green manuring with different varieties on the yield of wheat was studied by Khan and Mathur.[56,57] Green manuring was found to leave residual fertility in the soil even after two crops had been taken. The application of sunnhemp after composting was found to be a better practice. They further recommended that sunnhemp should be buried when about 8 weeks old and that when wheat is raised it should be sown 8 weeks later. Application of 15 lbs. per acre N and 80 lbs. per acre P_2O_5 in addition to green manuring were found to give higher yields.

Vyas and Desai [58] studied the influence of superphosphate application on the nodulation and fixation of atmospheric nitrogen through pea. The application of phosphate was found to give significant increase in the number of nodules even from the third week of the growth of pea, the effect becoming more pronounced with age both in the number and weight of the nodules. The nitrogen content of the crop as also the dry matter produced was greater with the phosphate treatments, the effect being observed from the third week of growth.

In continuing the work with superphosphate Vyas [59] studied the effect of indigenous phosphatic manures on atmospheric

nitrogen fixation in the pea. Bonemeal, Trichinodule and Singbhum phosphate were studied and all these forms were found to be almost as effective as superphosphate. While the Trichinodule and Singbhum phosphate were inferior to super they were being equally effective between themselves.

Acharya, Jain and Jha [60] studied the influence of growing phosphate fertilized berseem (*Trifolium allexandrium*) on the nitrogen content of the soil. In analysing the data from 10 years experiments conducted on Delhi soil, they found that the fertility as measured by nitrogen content is increased by growing berseem manured with phosphate. It is recorded that soil nitrogen is increased even when the above ground portion of the legume are cut and removed from the land, which is at variance with results recorded elsewhere. They reported [61] that growing of berseem in rotation with wheat led to the building up of soil fertility as shown by increase of organic matter from 0.696 to 0.989% over a 10 year period. The organic matter level was also found to be significantly higher in plots that had been treated with super (32 to 64 lb. P_2O_5 per acre), but that the treatments did not alter the C/N ratio of the soil.

Cultural operations: In an effort to find out the possibility of inter-cropping in sugarcane fields, Rege and Patwardhan [62] tried maize as the intercrop and found that this operation affects tillering and growth of cane and reduces yield. Additional application of 50 lbs. N at earthing up benefited crops where maize plants were 3 feet apart and this procedure is recommended. Closer planting of maize was found uneconomical. Kapoor, Handa and Chopra [63] described the cultivation practices of henbane (*Hyoseyanus niger* Linn). A well drained fertile sandy or silt loam is favourable for henbane farming. Broadcasting the seed gives better yields than transplanting from nurseries. A good crop required a well prepared soil and occasional irrigation and weeding.

Thakar [64] studied the effect of different crops in rotation with cotton and found that cotton following lang (*Lathyrus sativus*) was significantly superior in yield and quality to that grown after jowar and receiving farm yard manure.

Mukherjee [65] delailed the various aspects relating to the

cultivation, uses and future improvement of mango and reviewed the literature relating to the soil and nutrient requirements of this crop under Indian conditions.

Balasubrahmanyan [66] reviewed the results from the various experiments conducted on cotton in Madras. Removal of sorghum stubble immediately after harvest was found to improve the Karunganni Cotton yield by 10% in Coimbatore District. Mixed cropping of indigo and sorghum in another raised the yield of the cotton crop following by about 16%. In two districts N fertilizing was found profitable.

Mokashi and Khargaonkar [67] described investigations for sampling of yields from long term crop rotation experiments with cotton-jowar-wheat and jowar-groundnut-cotton-wheat sequences. Increase in standard error of estimated grain yields was lower for wheat than for jowar. Working out the costs it was found that the cost of sampling was justified for wheat but not for jowar.

Crop and land improvement methods under the Krishna East Bank Canal were discussed by Satyanarayana.[68] He found that temporary substitution of a second paddy crop for the pulses normally following paddy reduced soil alkalinity. The alkalinity of soils unsuitable for pulses could be reduced by flooding after paddy harvesting and growing green manure crops after the paddy on removing strips of surface soil. Where the slope of the land permits, trenches dug across the field to allow the free flow of drainage water into the main drains were found beneficial so long as water was supplied by the canals.

Khan [19] studied the effect of seed bed preparation with three different forms of implements on the yield of wheat. Power cultivation with tractor implements was compared with bullock cultivation with the Victory and Country ploughs. Soil inversion with the bullock drawn Victory plough gave markedly increased yields, while greater depth of cultivation secured by tractor implements was not conducive to bigger yields.

Farm refuse, domestic waste, etc., for manurial purposes: The results of experiments on the value of urine as a manure presented by Idnani [70] showed that a variety of waste materials, such as cotton

flue dust and saw dust can be used as absorbents of urine and the greater part of added N could be retained in them by quick drying in the sun. Treatments of wheat in pots with human urine manure proved superior to $(NH)_2SO_4$ applied at the same rate of N and increased grain yields by 140%. Joshi and Deshpande [71] studied the use of typical samples of silt from various sources in Madhya Pradesh, as bulky manure.

The utilization of human wastes like nightsoil and urine for conversion into manure by the adoption of a simple system of trenching was described by Choudhari [72] and the opportunities for its adoption in rural communities stressed.

Pandalai, Sankarasubramany and Menon [73] presented data on the composition of press mud cakes from different factories and suggested their use as manure and soil ameliorant for cocoanut soils. As a source of potash press mud cake did not compare favourably with ash or other potassic fertilizers.

The mineral composition of the ash of various oil seed cakes was determined and given by Prakash, Sharman and Khan [74] as an aid in determining their fertilizer value. The manurial resources in India from varieties of human, animal, agricultural and forest wastes were computed by Acharya [75] and the losses resulting from their non-utilization were reviewed.

Manurial practices and crop production : Ekbote and Kalamkar [76] presented the results of a large number of fertilizer trials conducted with wheat and paddy for over 10 years and discussed their significance. Except for an immediate increase of wheat production, various organic manures appeared to be superior to mineral fertilizers particularly in the long run. Farmyard manure and compost were more efficient than ammonium sulphate and sodium nitrate had decidedly harmful effects.

The improved manurial regime which gave increased paddy yields were discussed by Parthasarathy.[77] In general 4,000-5,000 lbs. per acre of green manure supplemented by 150 lbs of $(NH_4)_2SO_4$ and super were stated to increase the yields in India depending upon the condition of the soil. Thin sowing of paddy nurseries and good cultivation practices are essential for high yields. The methods of cultivation of rice in Japan were described and comparing with

Indian conditions, the heavy application of inorganic fertilizers to both the seed beds and transplanted fields in Japan are not advocated for this country.

The effect of nitrogenous fertilizers on the incidence of "blast" on rice varieties were discussed by Padmanabhan[78] and of the 9 varieties tested it was found that increase of foliar infection by *Piricularia oryzae* with increasing levels of N varied significantly between these varieties. With six levels of N ranging from 0-100 lbs. per acre there was no increase in infection in Co. 4 while in Co. 13 infection rapidly increased with each increase in level upto 80 lbs. It was suggested that resistance to different varieties should be conducted at the economically optimum level for the country, *viz.* 40 lb. per acre N.

The influence of soil nutrients on the growth of hyacinth (*Hyacinthus orientalis* Linn) were studied by Amin and Watson.[79] It was found that the nutrient solution containing 100 p.p.m. each of N, K and Ca, 50 p.p.m. Mg, 20 p.p.m. P and moderate levels of micro-elements produced plants having the longest flowering period and best quality and yet produced early flowering. The highest degree of uniformity occurred in plants grown in a low P concentration of 200 p.p.m. with moderate nutrient levels.

The method for applying fertilitizers to soils to obtain the best results were detailed by Raheja and Dhillon.[80] The importance of placement of fertilizer, the relationship to soil type and moisture conditions were stressed.

The need for manuring the cocoanut palm both by natural green manuring methods and application of artificials were stressed by Verghese.[81] Along with green manures (5,000-10,000 lbs.) the application of 0.25 lb. N, 0.5 lb. P_2O_5 and 0 75 lb. K_2O per palm were suggested. Chandrasekhara, Mathur and Das[82] studied the influence of rotation and fertilization on the quality of wheat and found (1) that fertilization with phosphates depressed the protein content, (2) inclusion of legume in rotation increased the protein in the grain; (3) that F.Y.M. did not increase thiamine content as compared to the control or the NPK plot.

Desai, Sundara Rao and Tejwani[83] from lysimeter studies of crop rotations concluded that cultivation of a legume like berseem

in the rotation improved soil productivity, phosphate manuring enhancing this effect. The influence of the above rotations and treatments on the quality of the crops were studied[84] and it was found that combined application of nitrogen and phosphorus to wheat resulted in most efficient utilization of fertilizer material while application of phosphorus alone resulted in inefficient utilization of fertilizer material. Introduction of legume in the rotation resulted in larger quantities of the nutrients being made available and absorbed by the crops.

Gopalan Nayar[85] studied the influence of manurial combinations on the yield of poovan banana and found that 0.25 lb. N as cattle manure and 0.25 lb. as $(NH_4)_2 SO_4$ in two doses applied over a basal dose of 25 lbs. of cattle manure was superior to other combination of nitrogenous manures. Under the heavy clay wet land soils of Aduthurai no response to addition of K or P was found.

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CHEMISTRY OF PLANT PRODUCTS

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The tempo of work in this useful field has been high during 1953. Oil Merchants' Chamber Limited, Bombay, held a symposium on *Oils, fats and allied products.*[1] A symposium on *Antibacterial substances from soils, plants and other sources*[2] was held under the auspices of the Pharmaceuticals and Drugs Committee of the C. S. I. R. The National Institute of Sciences held a symposium on *Indigenous drugs and insecticides.*[3] In December 1952, Indian Academy of Sciences held a symposium on *the scientific problems of the sugar industry.*[4] Successful attempts have been made to cultivate in Kashmir some of the medicinal plants not found in India.[5]

The work reviewed here has been classified under the headings: (1) oils and fats, (2) essential oils, (3) alkaloids and, (4) other plant products.

Oils and fats

Castor oil has been fractionated on the principle of liquid-liquid extraction to obtain tri-ricinolein.[6] The complex nature of the action of sulphuric acid on castor oil and its fatty acids has been studied.[7] Substituted sulphonic acids in small concentration are effective dehydrating agents for castor oil.[8] Gasoline, b.p. 60-100°C., has been found to be a good solvent for castor[9] and mahua[10] seeds.

The oil contents of whole seeds and kernels of nine varieties of Indian cotton seed ranged from 14 to 19.4% and 29 to 35% respectively.[11] Examination of seeds from Hyderabad and Bombay has indicated the existence of two different types characterized by high and low seed weights, oil contents and residual linter contents respectively.[12] On alcohol extraction the seeds yield 28.5% of oil.[13] Crude oil has been refined by successive treatment with sodium hydroxide solution and decolourising carbons or earths.[14] A general article by Smith deals with cottonseed fatty acids.[15]

Indian cotton seed oil-foots have yielded 50.9% saturated and 49.1% unsaturated fatty acids.[16] A mixture of the oil-foots and lime when pyrolised at 450-80°C. has given a crude oil (yield 21%; calorific value 17,400 B.t.u.) containing 31 to 35% gasoline (b.p. upto 200°C) and 45 to 50% kerosene (b.p. upto 325°C.).[17]

By low temperature crystallization and spectroscopic analysis, the seed kernel fats of *Cucurbita maxima*, *Benincasa cerifera*, *Langaneria vulgaris* and *Citrullus vulgaris* obtained in 48, 48.3, 45.1 and 69.4% yields have been shown to consist of: linoleic acid (43.7, 62.3, 64.0 and 58.6%), oleic acid (26.4, 21.9, 18.2 and 18.7%) and saturated acids (29.9, 15.8, 17.8 and 22.7%) respectively.[18]

Oil obtained in 33% yield from the seed kernel of *Zizyphus jujuba*,[19] (*Ber* in Hindi, *Bor* in Marathi) has the following characteristics and composition: Sp. gr. (Specific gravity) at 25°C.: 0.9117; R. I. (Refractive index) at 30°C.: 1.4631; A. V. (Acid value): 2.42; S.V. (Saponification value): 194.5; I. V. (Iodine value): 87.4; R.M.V. (Reichert-Miessel value): 0.62; P. V. (Polenske value): 0.88; oleic acid: 71.68%; linoleic acid 15.36%; solid acids: 12.96%, unsaponifiable: 0.81%.

From a study of its acetyl value, U. V. absorption and permanganate oxidation, *Kamlolenic* acid isolated from *Kamala* (*Mallotus philippensis*) seeds has been confirmed to be w-hydroxy 9 : 11 : 13. octadecenoic acid.[20] While ether, ethyl acetate or benzene extracts all the oil in *Kamala* seeds, petrol (b.p. 40 to 60°C.) extracts only a fraction.[21]

The characteristics and composition of the seed kernel oil[22] (7.5% yield) of *Tamarindus indica* are: Sp. gr. at 32°C., 0.9189; R.I. at 25°C.; 1.471; S.V., 190.4; I.V.: 118.2; unsap., 2.64%. acids: —palmitic, 6.19%; stearic, 2.64%; arachidic, 2.42% behenic, 6.86% oleic, 38.00% and linoleic, 41.34%.

Spectroscopic examination of *Tung* oil[23] from the tea gardens in North Bengal has indicated it to comprise α-eleostearic acid 66.1%; linoleic acid, 11.1%; oleic acid, 12.79% and saturated acids, 10.1%.

Karanja (*Pongamia glabra*) oil has been refined by cold extraction with methyl or ethyl alcohol.[24] The fatty acids of the

oil [25] are: myristic, 1.6; palmitic, 7.9; stearic, 3.7; arachidic, 2.5; behenic, 4.2; lignoceric, 1.1; oleic, 62.1; linoleic, 11.9 and linolenic, 5.0%.

Characteristics and composition of seed oil of *Moringa pterigosperma* [26] are: Sp. gr. at 30°C., 0.8984; R. I. at 30°C., 1.4652; S. V., 182.20; I. V., 64.20; R.M.V., 0.44; A. V., 3.48; acetyl value, 11.50; Hehner value, 91.59 and unsap., 3.05%; acids:—palmitic, 9.3; stearic, 7.4; behenic, 8.6 and oleic 65.7%.

Nutmeg-aril, *Jaypatri* (*Myristica fragrans*) has yielded to carbontetrachloride extraction a mixture of fatty and essential oils. The characteristics of this mixture and those of the fatty oil from the steam exhausted aril are: yield (26 and 18-20%) Sp. gr. (0.9884 at 31°C. and 0.9769 at 28°C.); R. I. (1.4850 at 35°C. and 1.4835 at 28°C); S. V. (108 and 161 to 162) and I. V. (153-7 and 118-119) respectively.[27]

Niger (*Guizota abyssinica*) seed oil [28,29] and safflower (*Carthamus tinctorius*) seed oil [30] are easily susceptible to oxidative rancidity.

Petroselinic acid is present to the extent of 21, 45 and 42% of the total fatty acids of the seed fats of (*Cuminum cyminum* (*Cumin*), *Anethum sowa* (*Dill*) and *Ptychotis ajowan* (*Ajowan*) respectively. The naturally occurring *cis*-acid and the corresponding *trans*-acid gave the lower and higher melting dihydroxy acids respectively, on peracid oxidation.[31]

Recent advances [32] and the topic of utilization [33] of wastes and byproducts of the oil and allied industries have been detailed in two general articles.

Alcohol has been used as a solvent for extraction of oils from oil-cakes and seeds.[34-36]

Component fatty acids in Dalda and Lion brands of hydrogenated oils have been determined.[37] Tyrosine ethyl ester has been suggested as a possible indicator substance for detection of adulteration of ghee with hydrogenated oil.[38] It has been indicated that peroxide number cannot always show conclusively the degree of deterioration suffered by oils on storage.[39] Added carotene has been found to be most stable in the hydrogenated oil and least stable in the refined oil.[40]

In a review article on *the aetiological role of toxic oils in epidemic dropsy*, characterization and effects of different adulterants in mustard seeds and oil have been discussed.[41]

Different aspects of alcoholysis of vegetable oils have been studied.[42-44]

Oil-seeds, when fumigated with calcium cyanide absorb and retain HCN beyond permissible levels and hence this procedure is not to be used for preservation.[45]

The property of double bonds to add on hypochlorous acid (HClO) has been utilized for estimation of unsaturation of fatty acids and esters.[46] Unsaturation of cashew nut shell liquid can be estimated by determining the iodine number of cardanol isolated from the liquid.[47] A micro-method for determination of yield and iodine value (Hanus) of oils from 0.3 to 0.5 g. of seeds has been described.[48] Hydrogenation iodine number (HIN) as a true measure of unsaturation of oils has been determined using Towers micro-hydrogenation apparatus.[49]

A process for the preparation of metallic salts of polymerised fatty acids has been described.[50]

Variation of glyceride type composition of specific fats under different environments has been studied.[51] An improved method for determining the GS_3 content of natural fats has been described.[52]

Aca-catechin (*Katha*) has been found to be an efficient antioxidant for groundnut oil.[53] A method to demonstrate the presence of different antioxidants in fats and oils, has been developed.[54] With groundnut oil as substrate, the rate of destruction of antioxidants from licorice root (*Glycyrrhiza glabra*) follows the unimolecular reaction rate.[55]

Some aspects of the biosynthesis of specific fats under different environments have been investigated.[56] Chemistry of odour and colour imparting substances of oils and fats have been described.[57] Earths from Hyderabad have been tested for their bleaching properties.[58] Castor, safflower and linseed oils have been hydrolysed to the extent of 89% within 24 hours by lipolytic enzyme of castor beans.[59] Oil varnishes containing cashew-nut shell liquid-rosin condensate are superior to those with ester gum and

zinc rosinate.[60] A review article containing information on the occurrence, composition, extraction and refining of sugar cane wax has been published.[61]

Essential oils

Characteristics of guava *Psidium guayava* leaf oil from Allahabad [62,63] and Bangalore[64] varieties are: yield (0.31% and 0.26%); Sp. gr. (0.9192 at 27°C. and 0.9001 at 26°C.); R. I. (1.4870 at 23°C. and 1.4882 at 25°); Optical rotation (—13.06; —27.4 and after six months +5.5) and ester value (4.7 and 12.3) respectively. Both contain *d*-as well as *dl*-limonenes, two sesquiterpenes and a sesquiterpene alcohol. Bangalore variety contains β-caryophyllene also.

Presence of *d* α phellandrene in *Bel* (*Aegele marmelos*) oil has been established.[65]

Leaf oil from *Premna tomentosa* [66] has the following characteristics and composition: yield, 0.073%; Sp. gr. at 31.6°C., 0.8740 R. I at 29°C., 1.4862; optical rotation, +31.44; ester value, 8.9; acetyle value, 14.9 and I.V., 75.1; Limonene, 57.8%; β caryophyllene, 17.2%; sesquiterpenes, 7.8%; sesquiterpene alcohol 5.6%; and diterpene, 5.5%.

Bellary leaf (*Litsea zeylanica*)[67] oil contains ocimene (35%), α terpinene (20%), dipentine (5%), p-cymene (5%), aromadendrene (2%) alcohols (25%) and residue (8%).

Clove buds and stems from Travancore have yielded 16% and 7% of clove oil containing 97% and 95%, respectively of eugenol.[68]

Characteristics of nut-meg (*Myristica fragrans*) leaf oil, (yield 0.5%) are: Sp. gr. at 30°C, 0.8642; R.I. 1.474 and ester value, 8.44. It possesses phytocidal action.[69]

Oil from the berries of *Juniperus communis*, has the following characteristics: yield, 0.83%; Sp. gr., at 27°C. 0.9180; R.I. at 25.5°C., 1.4820, optical rotation, +20.8; acid value, 4.7, ester value 20.5. Its major constituent is *d* - α - pinene.[70]

Characteristics of oil from the seeds of *Seseli indicum*[71] are: yield, 2.5%; Sp. gr. at 32°C., 0.8905; R.I at 32°C., 1.4821; optical rotation, +34.7; acid value, 3.1; ester value, 30.0; S.V.

33.1 and S.V. after acetylation, 64.6.

Otto of *Bela* or *Moghra*[72] (*Jasminium sambac*) has the following characteristics: Sp. gr. at 30°C., 0.9727 to 0.9797; R.I. at 30°C., 1.506 to 1.507; A. V., 1.5 to 11.4; ester value, 121 to 132; S.V. after acetylation, 229-242.

During studies with oil of *Pinus longifolia* it has been shown that two directional fission of the cyclopropane ring in 3-carene occurs on sulphur dehydrogenation yielding p- and m-cymenes.[73] 3-Carene has been isomerized to α-terpinene by refluxing with oxalic acid for 42 hours.[74]

Possibilities of utilizing Indian turpentine oil and rosin as raw materials for fine chemical industry have been discussed.[75]

Lemon grass oil produced in Uttar Pradesh compares well in quality with the East Indian variety produced in Travancore.[76]

Rectified and de-mentholised oil from *Mentha aravcnsis* is as good as the oil from *Mentha piperita*.[77]

General articles on Indian (Mysore) linaloe oil [78] and *Ylang-ylang* oil[79] have been published.

Alkaloids

Four alkaloids, two water-soluble [80]: (*prurienine* m. p. 263-264°C. decomp., and *prurieninine*, m. p. 213-14°C.) and two water-insoluble [81] (*mucunine*, m. p. 220-221°C. and *mucunadine*, m. p. 201-202°C.) have been isolated from *Mucuna pruriens*.

Skimmianine, $C_{14}H_{13}O_4N$, m. p. 176-77°C, three coumarins and *laureoline*, m. p. 250-60°C. have been isolated from the leaves and bark of *Skimmia laureola*.[82]

Berberis lamberti [83] has yielded oxycauthine, berbamine, palmatine, jatrorrhzine and columbamine and two new bases provisionally named *lambertine*, $C_{20}H_{19}O_4N$, m. p. 163°C. and *berlambine*, $C_{20}H_{17}O_5N$, m. p. 199-200°C.

While Chatterjee and Majumdar [84] have claimed the isolation of a new alkaloid *glycosin*, $C_{15}H_{12}ON_2$, m. p. 155-56°C. from *Glycosmis pontaphylla*, and advanced a 4-quinazolone structure.[85] Chakravarti and Chakravarti [86] as well as Chakravarti *el al.*[87] have maintained that the plant is *Glycosmis orborea* and *glycosin* is same as *arborine* isolated from *G. arborea*.

Formation of *yobrine*, $C_{19}H_{16}N_2$, m. p. 208°C. as the major product during selenium dehydrogenation has been adduced as direct evidence for the structure proposed by Chatterjee and Pakrashi [88,89] for *rauwolscine*. Das Gupta [90] has however indicated that some more chemical points have to be clarified before the structure could be settled.

Total alkaloids present in *Ephedra* have been estimated by steam distillation.[91] A chromatographic method (alumina column) for estimation of quinine and strychnine in pharmaceutical preparations has been described.[92]

Turpentine is a better solvent than kerosene, alcohol or dilute sulphuric acid for extraction of alkaloids from nux-vomica.[93]

Stoll [94] and Chowdhury [95] have described the cultural, chemical and therapeutical aspects of ergot.

Copper and zinc contents of roasted coffee powder and its decoction have been estimated.[96] Based on the fact that chicory contains inulin which on hydrolysis would yield levulose, a colorimetric method has been developed for detection of small amounts of chicory in coffee.[97] Caffeine, tannin and fibre contents of Indian tea have been studied.[98]

Chemical changes that take place during flue-curing and their bearing on the quality of leaf of cigarette tobacco have been studied.[99] Nicotine in tobacco has been estimated by semi-micro method of steam distillation followed by spectroscopic determination.[100] Processes for recovery of nicotine sulphate from tobacco wastes have been detailed.[101,102]

Cultural studies have shown that cultivated variety of henbane plants (*Hyocyamus niger*) improve in their alkaloidal content.[103]

Other plant products

Artemisia maritima growing in Kashmir contains 1.2% of santonine.[104]

Bitter principles of root-bark [105] and trunk-bark [106] of *nim* have been isolated.

Two colourless crystalline substances, *centipeda I*, m. p. 241°C., and *centipeda II*, m. p. 64°C, have been isolated from *Centipeda orbicularis*.[107]

A new aldehydo-lactone, m. p. 43-44°C., b. p. 213-16°/ 7 mm. has been isolated from the seed kernel of *Holygarna arnothina*.[108]

Investigation of 19 species of Indian *Dioscorea* has shown that diosgenin contents of *D. prazeri* and *D. deltoidea* are exceptionally high waranting exploitation.[109]

Corchorgenin, m. p. 237°C. a bitter genin isolated from defatted seeds of *Chorchorus olitorius* shows digitalis-like cardiac action.[110]

Muricatin A isolated from *Ipomoea muricata* seeds gives *muricatin B* on alkaline hydrolysis. From a study of the aglucone $C_{18}H_{34}O_4$ from *muricatin B*, tentative formulae for *muricatins* A and B have been suggested.[111]

An attempted synthesis, of *Kutkin*[112] isolated from *Picrorhiza kurrooa*, has indicated that the tentative formula suggested earlier [113] for *Kutkin* would require modification.

Isolates from *Tephrosia lanceolate* have been shown to be toxic to fish. [114,115]

Decussatin, $C_{16}H_{14}O_6$, m. p. 149-50°C. and *swertinin* $C_{15}H_{12}O_6$ m. p. 217°C., isolated from *Swertia decussata* have been characterized as 3' : 4 : 7-trimethoxy-8-hydroxy xanthone and 3 : 4-dimethoxy-8-hydroxy xanthone respectively.[116,117]

Scoparone,[118] $C_{16}H_{20}O_5$, isolated from *Artemisia scoparia* and previously known as *scoparine* [119] has been characterized as a keto-dimethoxy-methyl-octahydronaphtha coumarin.

Paper chromatography has been employed for a survey of anthoxanthins [120,121] and anthocyanins [122] among plant materials *Iso* flavones of soya beans have been confirmed as genistein and daidzein.[123] Anthoxanthins of two varieties of celery seeds have been examined.[124] 3 : 5 : 7 : 4' : 5'-Pentamethyl-3' ethyl ether of myricetin from *Hibiscus cannabinus* has been synthesised.[125] *Tetuin*, glucoside from seeds of *Oroxylum indicum* has been shown to be baicalein-6-glucoside.[126] Two crystalline glucosides identified as *divaricosid* and *caudosid* have been isolated from seeds of *Strophanthus whightianus*.[127]

A species of Indian *Parmelia* lichen contains lecanoric acid, atranorin and lichexanthone.[128] Usnic acid and barbatic acid[129] from *Usnea longissima* prevent growth of tubercle bacilli.[130]

Morellin, $C_{24}H_{29}O_6$, m. p. 168°, has been isolated from the seed coat of *Garcinia morella*.[131]

Saponins from soapnuts have been isolated by fractional precipitation of their aqueous extract with ammonium sulphate.[132]

Jeolic acid from *gum jeol* (*Lanna grandis*) behaves like arabic acid.[133-135] Aldobionic acid, a degradation product of jeolic acid is non-colloidal in character.[136] Gum jeol (*Odina wodier*) exhibits higher viscosity than the *gum-acid*.[137]

Leaves of drumstick (*Moringa pterygosperma*), sesbania (*Sesbania grandiflora*) and groundnut (*Arachis hypogaea*) contain 8.2, 8.2 and 9.4% protein on wet basis and 27.3, 34.6 and 35.2% respectively on dry basis.[138] Paper chromatography has indicated the presence of leucine, phenylalanine, tyrosine, alanine, arginine, histidine, lysine and cystine in tomato fruit (*Lycopersicum esculentum*).[139] Threonine content of Bengal gram is about 4.05 g. per 100 g. of protein [140] and not as reported earlier. [141] Amino acids of Bengal almond [142] wheat gluten and mango seed kernel [143] have been identified by paper chromatography. Chowdhury's method [144] for estimation of nicotinic acid in wheat gives results comparable to those obtained by other methods.[145]

Stability of ascorbic acid in *neera* from date palm (*Phonix silvestres*) has been studied.[146]

Water soluble carbohydrates of *Agave vera cruz* have been detected by paper chromatography.[147,148] Qualitative make up of water soluble carbohydrates of *Fureroea gigantea*,[149] *Alluim cepa* and *Alluim sativum* [150] is similar to that of *Agave vera cruz*.[151] Acid hydrolysis of tapioca starch has yielded a product containing 70% glucose and 28% maltose.[152] Levulinic acid has been prepared from mola ses by treatment with HCl.[153] Xylose has been isolated in 10.8% yield from jute stalks.[154] Cane sugar can be oxidised to tartaric acid using nitric acid as oxidising agent and vanadium as catalyst.[155]

Oxidation of jute-holocellulose with nitrogen dioxide has been studied.[156] Lignin contents of rice husk, betelnut husk and bamboo have been determined by different methods.[157] Degree of polymerisation of cellulose nitrate from standard Indian cotton seems to be fairly high.[158] Cellulose acetates from long staple

Egyptian cotton [159] differ from those of short and medium staple Indian cottons.[160]

Ulla grass [161] and varieties of woods [162-167] have been examined as possible indigenous cellulosic raw materials for the production of pulp, paper and board.

Ash analysis [168] and destructive distillation [169] of some hard wood species of Bombay State have been carried out.

Dalbergin, $C_{10}H_{12}O_4$, a lactone from *Dalbergia sissa* wood has been characterized as 3-phenyl-aesculetin methyl ether.[170]

Three isomeric compounds of formula $C_{10}H_{12}O_2$ isolated from *Thuja plicata* have been characterized as thujic acid, γ-thuja-plicin (γ-*iso*propyl tropolone) and β-thujaplicin (β-*iso*propyl tropolone).[171]

Destructive distillation of coconut shell at the lowest possible temperature yields the most active charcoal.[172]

Evaluation[173] of vegetable tanning materials has been carried out by the rapid Schultz method.[174] Study of potentiometric titration curves,[175] as well as acid salt ratio [176] of Indian vegetable tanstuffs has indicated the possibility of substituting wattle by mangrove or white-valem bark Hide powder method has been adopted for preparation of pure tannin from Avaram bark. [177]

Preparation and application of chlorophyll and copper chlorophyll have attracted considerable attention this year. [178-181]

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BIOCHEMISTRY OF SANITATION WITH SPECIAL REFERENCE TO WATER, SEWAGE AND INDUSTRIAL WASTES

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The first six volumes of the *Annual Review of Biochemical and Allied Research in India* (1930-35) contain accounts of certain aspects of sanitation, including water and sewage.[1-7] Volume XI of the *Annual Review* (1940) contains another account of the 'Chemistry of sewage and water purification'.[8] An account of 'Industrial wastes disposal' was published in the *Review* for 1950 (Vol. XXI).[9] The present account therefore includes the papers on water treatment and sewage purification published since 1940 and also the publications on industrial wastes since 1950.

With the rapid increase of population and of industry in recent years, the problems of water supply and treatment of sewage and industrial wastes have become increasingly difficult, and the necessity for a Central Water Pollution Research Laboratory for more intensive study of these problems has therefore been emphasised.[10-12] The work done during the period under report may be briefly summarised as follows

WATER

Topographical considerations: Bose[13] carried out a chemical examination of water samples from tube-wells in and around Calcutta. Although the water was found satisfactory by bacteriological tests, it was very hard and unsatisfactory for cooking. The water in some of the wells contained sufficient sodium chloride to impart a brackish taste. On the basis of the composition of the well waters, the district was divided into four divisions. Krishnamurty[14] described the geology of water resources of parts of Mahaboobnagar and Gulbarga districts of Hyderabad.

Subrahmanyan *et al.*[15] presented the results of their studies carried out during 1944-46 on the quality of water supplies drawn

from tube-wells (254), shallow open wells (55), and tanks (4), of the rural districts of the Singur and Balarambati Union Boards in West Bengal, and discussed their significance in drawing up standards for rural water supplies from the bacteriological point of view.

Chari [16] pointed out that only 20% of India's population had any system of water supply, the remaining section depending upon small lakes and wells for their water supply. With the spread of rural electrification, however, small water pumping stations are being built in many villages. At the same time, during the summer months there has been frequently a great shortage of water in the large cities. The corporations of Bombay, Madras, Calcutta and Delhi have launched schemes in order to increase their water supplies. For example, the water supply for the City of Bombay [17] was formerly obtained from 3 lakes, Tansa, Vihar and Tulsi, giving a total dependable supply of 79 m. g. d. This is now insufficient to meet the growing demands, and a scheme is being developed to impound water from the Vaitarna river and carry it to Tansa lake through a tunnel. This would give a total dependable supply of 175 m. g. d.

Studies on waters around certain urban areas and villages: Rao [18] drew attention to the pollution of the Halla river at Mandya (Mysore State) caused by the waste waters from a distillery using cane molasses.

In a study of the sources of drinking water supplies, particularly in regard to infestation with cyclops, the intermediate host of guinea worm, in the Osmanabad district of Hyderabad, Rao [19] observed that the incidence of guinea worm disease for the whole district was 28.9 per 1,000 and in some villages it was as high as 155 per 1,000. In all villages where the incidence of the disease was over 100 per 1,000 the water supply for the whole village was obtained from one step-well. Addition of lime to well water at monthly intervals, particularly between August and April when the disease was at its maximum, was suggested as a temporary measure. As a permanent measure against the infection provision of central water supplies for the larger towns and construction of draw wells of standard design for villages were recommended.

In a limnological investigation and improvement of villages, Biswas [20] observed that if the self-purifying action of surface waters in India was to be maintained, the surface vegetation should be controlled and the rooted water weeds and algae (which would provide food for fish) should be allowed to develop. In an account of water weeds and their eradication from irrigation canals in the Bombay Deccan, Inglis and Gokhale [21] suggested that if the canal was designed to maintain a velocity slightly above that at which no silt was deposited and no scouring action occurred, growth of weeds could be prevented by the construction of a pick-up weir, which would make the water turbid. Turbidity would retard the growth of plants by preventing access of light.

Bal and Pradhan [22] and Bal *et al.*[23] described the planktonic organisms and the chemical and physical conditions in the waters of Bombay harbour. Jayaraman [24] collected data on the seasonal variations in the salinity and concentrations of dissolved oxygen, phosphate, nitrate, nitrite and silicate in sea water of the Bay of Bengal off the coast of Madras during 1948-49. Seshappa [25] made some observations on the composition of mud banks along the Malabar coast and on the seasonal changes in the concentration of phosphate in the mud in relation to the concentration of phosphate in the overlying water and to the seasonal variations in the biology of the area. Roy's [26] studies on the planktonic organisms in the Hooghly river over a period of three years showed that variations in the numbers of phytoplankton, particularly diatoms, were mainly responsible for annual variations in the yield of plankton.

Chopra *et al.*[27] reviewed the literature on the action of pyrethrum on mosquito larvae and gave results of their laboratory experiments and field studies on the subject.

Methods of detection of water pollution : The occurrence of the *aerogenes* group of coliform organisms in faeces and its significance in water analysis were studied by Raghavachari and Seetharama Iyer [28] who carried out experiments in order to determine if *aerogenes* organisms isolated from faeces would grow in McConkey's broth when incubated at 44°C. Thirty-one strains out of a total of 478 organisms which fermented lactose belonged

to the *aerogenes cloacae* group I, according to the Ministry of Health's scheme of classification. Of these, 24 grew consistently in McConkey's broth at 44°C. and the remaining 7 did not grow at 44°C., and each of these sub-groups retained this characteristic after repeated sub-culture. As a result of their studies on *B. aerogenes* in the faeces of persons suffering from intestinal infections and its significance in water analysis, Krishnan and Chawla [29] reported that *aerogenes* should be seriously considered as an indication of faecal pollution. Raghavachari [30] defined the terms, "safe", "potable", "polluted" and "contaminated" as applied to water. He stated that the reactions of coliform bacteria to certain tests which are specific in temperate regions are different in the tropics. Generally the substances contained in water are too small in amount to have any physiological effect and, except when some absorbable poison is present, water for drinking requires only bacteriological purification. Palatability and cooking properties are good guides in the selection of a source of supply of drinking water.

Pasricha and DeMonte [31] considered bacteriophage as an index of water contamination and described a method of examining water for faecal contamination based on the assumption that such contamination is indicated by the presence of bacteriophages against pathogenic organisms in human intestine.

In a series of communications by Dyer, [32,33] Dyer and Bhaskaran,[34-36] Dyer *et al.*[37] and Subrahmanyan and Bhaskaran [38] is given information of practical interest on the spread of ground water pollution from bore-hole latrines or leaching cesspools. The extent of pollution of ground water was found to depend chiefly on the velocity of flow of ground water which in turn depended on the physical structure of the soil and on the gradient of the water table. The safe distance from a source of pollution might be taken as the distance through which ground water would flow in 8 days. In quick sand, pollution might spread to a distance of 260 ft. in the direction of flow of sub soil water in four months. Sodium chloride might be used successfully to indicate the direction and rate of flow of ground water in acid and neutral soils; the results obtained by this

method in alkaline soils were not always reliable. The studies on ground water pollution in West Bengal showed that shallow wells in sandy soil might be satisfactory hygienically as a source of water supply.

Seal and Banerjea [39] observed that the chief gastro-intestinal disorders in groups of persons using different waters in a rural area of Bengal were diarrhoea and dysentery, with cholera contributing to the morbidity rate only to a minor extent. Direct comparison of incidence of gastro-intestinal disorders with sources of drinking water supply or with their *coli aerogenes* content failed to show any evidence of correlation, but it was pointed out that disorders might arise from drinking from contaminated sources such as tank water.

Roy [40] indicated the complex nature of the problem of maintaining an abundant supply of wholesome water and how closely the microscopic organisms in water are associated with the health, hygiene and utility in the day to day life of man.

Bacteriological examination of water : Neogi [41] analysed 40 waters in Bengal in order to study the significance of the types intermediate between *B. coli* and *B. aerogenes*, and considered that Levine's E. M. B. agar (eosin-methylene-blue agar) test, while not strictly reliable for the bacteriological examination of water in Bengal, might be conveniently used as a test for partial confirmation of coliform organisms.

Pasricha *et al.*[42] described a " dilution method " for isolating pathogenic bacteria from faeces. The greater percentage of positive results obtained on plates inoculated with diluted suspensions of stools than by direct plating of samples of the stools might be due to breaking up of clumps of pathogenic bacteria during dilution. Swaroop [43] found that dilutions of 1/2, 1/10 and 1/100 were most useful in determining the most probable number of bacteria in 100 ml. of water, and calculated the most probable number and standard error in determination when 2, 3, 5 or 10 tubes were used for each of the dilutions.

Bhaskaran *et al.*[44] devised a simple field kit similar to the Horrocks outfit but incorporating equipment for the o-Tolidine test for determining the amount of chlorine required for disinfection of

a water. *o*-Tolidine reagent was found ten times more sensitive than the cadmium iodide-starch indicator of the Horrocks outfit. The minimum dose with the new outfit is 3.6 p.p.m.

Benerjea [45] made a comparative study of Voges-Proskauer test and Barritt's modification of this test (*J. Path. Bact.*, 1936, **42**, 441) for the differentiation of coliform bacteria, incubating 20 strains of *B. aerogenes* at 37°C. in glucose-peptone medium and in standard glucose-phosphate medium for 1, 2, 3 and 4 days His observations showed that the Barritt test was more sensitive than the V. P. test.

Tyagaraja [46] reported a simple modification of Wilson and Blair's medium for the isolation of typhoid and paratyphoid bacilli. Panja and Ghosh [47] carried out a comparative study of Wilson and Blair's medium and D. E. C. medium (a modification of Difco S. S. agar medium as described by these authors in 1943 [48]) for the isolation of *B. typhosus*, and pointed out the advantages of the latter medium. This medium was first developed for isolating from faeces organisms causing dysentery, cholera and other enteric diseases.

Panja [49] described a method for isolating vibrios from cholera stools, soil, water and broth cultures of bacteria, using a porcelain candle in a mixture of peptone water and boric acid and drawing a little of the sample through the candle into the surrounding medium. Employing the candle-boric-peptone water method Panja and Ghosh [50] showed that 16 out of 524 samples of water from the Hooghly river at Calcutta contained *Vibrio cholera*. Other methods of examination detected far fewer vibrios. It would also appear that the use of unfiltered river water might be a cause of cholera. Dudani *et al.*[51] reported their preliminary observations on the dehydrogenase activity of a strain of *Vibrio cholera* and of variants of the strain (see p. 79 of this volume).

Bose and Chakraborty [52] observed that metallic copper in the form of copper foil or copper containers had a bactericidal action on *Vibrio cholera*, *B.dysenteriae*, *B.typhosum* and *B.coli* and that the effect was more rapid in distilled water than in water containing organic nitrogenous substances. The observations of Karunakaran and Pillai [53] showed that copper, silver, brass, and tin-coated

copper vessels had a bactericidal action against bacteria present in water and other liquids stored in them, *e.g.*, *Salmonella typhi* in concentrations of 120 millions per ml. was killed within 30 minutes in silver vessel. This bactericidal property was considered chiefly a surface action and to a significant degree colloidal in character transferable to the medium and potent and stable when the medium was removed from the vessel. Gold appeared to favour the survival of bacteria for longer periods than glass.

Mention may be made in this connection of the work done at certain centres on the preparation of agar [54] and treatment and re-utilisation of used agar media [55,56] and McConkey broth.[57]

Sampling and chemical analysis of water: Raisinghani [58] suggested an improved type of bottle sampler for taking samples of running water for determination of suspended silt, in which a float would rise as the water entered the bottle and make contact with an electric circuit when the bottle was full.

Sen [59] proposed a method of estimation of total salts of soil extracts and waters based on the assumption that acid and basic radicals of a salt or a mixture of salts usually remain in the ratio 2 : 1. The sum of acid radicals CO_3, HCO_3, SO_4, Cl, NO_3 when multiplied by 1.5 would give the total amount of solutes in solution. There was fair agreement between analytical figures and calculated values.

Misra *et al.*[60] recommended a new reagent for detecting the nitrite ion, even as little as 1 γ of NO_2^-, using an aqueous solution of 2-amino-4-(chloromethyl) thiazolyl-HCl which would give a red colouration or a red precipitate with NO_2^-. The reaction would take place instantaneously.

Ramakrishnan [61] reported a modified method for the determination of magnesium in water in the presence of calcium. Excess of 8-quinolinol could be used ; this reagent would react with ferric chloride to give an intense green colour which would be stable and could be measured in a Duboscq colorimeter. Calcium should be precipitated with tungstate instead of oxalate as the latter would interfere in the reaction between 8-quinolinol and ferric chloride.

Roy [62] recorded that removal of temporary hardness of water

by boiling caused a decrease in electrical conductivity (F) which was proportional to the temporary hardness (T). A mean value for F/T of 16.6 was obtained for about 100 samples of water. By using this F/T ratio, close correlation with the soap method was obtained for temporary hardness of water samples.

Majumdar and Ray [63] studied the factors affecting the results when Willard and Winter's method was used for determining fluorine. They observed that the titre of the solution of thorium nitrate was affected by the pH value and the amount of alcohol present during the titration and that best results were obtained in the presence of excess alcohol and at a pH value of 2.34. Before testing the water for fluorine the water should be treated with calcium hydroxide (2% by volume), evaporated to dryness, and then treated in the usual way.

Rao and Venkateswarlu [64] modified Talvitie's method for the determination of fluorine in water by substituting $(NH_4)_2SO_4$ for Na_2SO_4 to prevent precipitation of thorium in the preparation of the reagent and by using acetate instead of formate buffer to give a more intense colour. The optimum pH was found to be 3.6 Venkateswarlu and Ramanathan [65] examined the various methods of determination of fluorine in potable waters for their adaptability to rapid estimation in the field, and they selected the salt-acid-thorium volumetric procedure of Williams as adapted by Smith and Gardner (Fluoride Studies, Atomic Energy Commission, Unclassified Document UR-36) with a few modifications.

Wadhwani [66,67] studied the factors affecting the thorium nitrate titration method for fluorine estimation, *e.g.*, the causes of variations in the reactions between thorium salt and hydrofluosilicic acid and the permissible concentration of different interfering ions, and described the conditions for accurate estimation of fluorine. He [68] compared the accuracy of this method with that obtained by the photo-electric method which is based on the bleaching effect of fluorine on the colour produced as a result of reaction between ferron and ferric chloride. He [69] also described a macro-determination of fluorine based on the reaction between hydrofluosilicic acid and thorium nitrate, resulting in the formation of H_2ThF_6.

Ramakrishnan and Venkataramanan [70] suggested that a test be made for saccharic acid as an interfering agent in Sanchis' test for fluorine when vegetable carbons are used for removal of fluorine from water.

Studies on settling tanks, sand filtration and chemical treatment of water for potable purposes: Subrahmanyan and Majumder [71] carried out a study (similar to that of H. W. Streeter, *U. S. Publ. Hlth. Bull.*, 1927, No. 172, Government Printing Office, Washington) of the efficiency of settling tanks in the water purification plants at Calcutta and Bangalore. The efficiency of bacterial reduction was found greater in the Calcutta settling tanks than at Bangalore, possibly because of the longer detention time. The old settling tanks of masonry in Calcutta were somewhat more efficient than the new settling tanks of earth, particularly in reduction of coliforms. The reduction of coliforms was found to take place at a higner rate than the reduction of the total count of bacteria on agar. Chemical and bacteriological analyses made by Ghosh [72] of waters which had been passed through filters containing sand of various sizes showed that the effective size of the medium for slow sand filtration was between 0.25 and 0.35 mm.

The experiments of Mitra [73] at the Pulta Water Works, Calcutta (having a capacity of 99 m.g.d.), during 1940-42 showed that in most cases raking the surface of slow sand filters was not followed by deterioration in the bacteriological quality of the filtrate, even though the rate of filtration was increased.

Camp, Root and Bhoota [74] described experiments on the effect of temperature on the speed of formation of floc in water by iron salts. The results indicate that provided coagulation takes place at the optimum pH value, temperature has little effect on the time required for formation of floc, but that the optimum pH value is affected by changes in the temperature and by the coagulant used.

Banerjea [75] found that disinfection of water with potassium permanganate was not significantly affected by the pH value of the water, but was retarded by the presence of more than 1 p.p.m. of organic matter.

In the annual reports of the Water Analyst of the Corporation of Madras [76] for the years 1945-50, information is given on the treatment and analysis of the water supplies of Madras. The water was chlorinated before and after the passage through shallow sand filters.

Incrustations in water distribution pipes: In continuation of the earlier studies on the tubercular incrustations in water pipes (S. C. Pillai, *Proc. Nat. Inst. Sci. India*, 1938, **4**, 295) further observations on the growth and activity of a species of filamentous iron bacterium (*Leptothrix ochraceae*) in a certain iron-bearing water in relation to the incrustations in the distribution system were carried out. [77] The experiments of Pillay and Kaimal [78] on the operational practice and tuberculation of pipes confirmed the earlier observation of Pillai (1938, *loc. cit.*) that the formation of ferruginous tubercles could be checked by treating the water with lime and raising its pH value to about 9.

Swaminathan [79] discussed the economic consequences of corrosion of iron and steel structures and surveyed some methods for reducing losses.

Studies on fluoride-bearing waters: Raghavachari and Venkataramanan [80] examined samples of protected and unprotected water supplies in the Province of Madras. Concentrations of fluorine over 3.6 p.p.m. were found in the Nellore district and in some isolated supplies; many supplies contained 1-3 p.p.m. fluorine. The content of fluorine and incidence of fluorosis were related to broad geological divisions. A further study [81] of the factors involved in the production of mottled enamel in children and severe bone manifestation in adults in certain areas in the Nellore district (where the fluorine contents of waters were high) showed that mottled enamel was always present where the water used contained more than 1 p. p. m. fluorine; that severity was in proportion to the content of fluoride, and deciduous teeth were sometimes affected in areas where the fluorine content of water was very high (over 6 p.p.m.); that symptoms of chronic fluorine intoxication occurred in adults only after residence for 15 years in the district; and that the incidence and severity of the disease were related to the economic and nutritional status of the communities, their

occupations and deficiency of vitamin C in the diet. The experiments carried out by Pandit and Rao [82] in this connection with monkeys indicated that the animals kept without vitamin C showed greater absorption of fluorine and greater excretion in urine than those which had vitamin C. Wadhwani [83] reported that vitamin C played an important ameliorative role in fluorine poisoning in monkeys.

Further to the earlier observations (S. C. Pillai, *Indian Med. Gaz.*, 1938, **73**, 408) on mottled enamel endemic in a certain locality in Nagercoil, Travancore, experiments were carried out with albino rats. The rat incisors were found peculiar in that the external symptoms of mottling as caused by fluoride disappeared gradually and almost completely consequent on the change over to fluoride-free water. The remarkably fast rate at which the incisors in rats generally grow would appear to have an important role in the mechanism of disappearance of the mottled symptoms: *e.g.*, when one of the incisors of a four months' old rat was removed, it was replaced to the full size in about six weeks' time. In regard to the treatment of the water, it was observed that addition of calcium hydroxide to water in quantities calculated to precipitate the fluoride did not seem to be efficacious in checking the incidence of the mottled condition of rats. Inclusion of sufficient amounts of whole milk powder (Klim) in the diets of experimental rats afforded remarkable protection against fluorine poisoning in the animals. A supplement of bone powder also brought about considerable relief from fluorine intoxication in the experimental animals.[84-86] It is of interest in this connection to note that the fluorosis of cattle in certain parts of Madras Presidency was cured by feeding the animals daily with 1 to 2 oz. of sterilised bone meal for a period of 6 months to 2 years.[87] The problem of mottled teeth in Nagercoil has been solved by change of water supply since 1945.[88]

In the rural areas of the Punjab, Shourie [89] found a correlation between the degree of mottling and the content of fluorine in drinking water and a considerably lower incidence of caries in children with mottled enamel.

Venkataramanan and Krishnaswamy [90] gave evidence that

addition of aluminium sulphate to the diets of young albino rats receiving 1 mg. F/day markedly inhibited the onset of severe dental changes like pitting and erosion. Venkataramanan *et al.*[91] described the preparation of a cation exchange resin from Avaram (*Cassia auriculata*) bark and its use (a two-bed system of the resin) with "Amberlite IR-4" as the anion exchanger in removing fluorine from water containing upto 5 p.p.m. It was further observed that treatment of the cation exchanger with alum conferred advantages of a one-bed system easily regenerated with alum.[92]

Wadhwani and De[93] reviewed the literature on fluorine poisoning. In a study of the mechanism of fluorine removal by calcium salts Wadhwani[94] observed that removal of fluorine from sodium fluoride solution by calcium phosphate, raw bone meal and purified bone meal followed the Freundlich adsorption isotherm. Rock phosphate, fluorapatite and chlorapatite did not remove fluorine. Calcium carbonate removed fluorine above concentrations of 3-5 p.p.m., but the percentage adsorption decreased with fluorine concentration. Calcium oxide and sulphate removed fluorine from sodium fluoride solution by a mechanism of anion exchange wherein complete fluorine removal was found limited by the solubility of the precipitated calcium fluoride. From their studies on the removal of sodium fluoride from aqueous solution (2-8 p.p.m.) by treatment with varying amounts of tricalcium phosphate, Ramakrishnan and Venkataramanan[95] inferred that the reaction was complex, involving anion exchange of F^- for PO_4^-, total molecular absorption of NaF, and possibly the partial replacement of Ca by Na in the phosphate. Ramakrishnan *et al.*[96] prepared an ion exchange material from coffee husk by suitable treatment with sulphuric acid, sodium chloride and alum. The material thus prepared was found considerably efficient in removing fluorine from tap water to which sodium fluoride (about 2.5 p.p.m. as fluorine) had been added.

Venkateswarlu and Rao[97] examined 173 samples of water from dug wells, bore wells, springs, river beds and upland surface waters from 39 localities in the Visakhapatnam area (Andhra State) and found that waters from 3 localities contained less than 0.5

p.p.m. of fluorine, those from the majority of the localities contained about 1 p.p.m., those from a few localities contained higher amounts, and those from the dug wells in one locality contained 11 p.p.m. of fluorine. The wide variations in the fluorine content of waters belonging to the same locality appeared to depend on the fluorine contents of the rocks and soil percolated by the waters. In an examination [98] of 1,000 children aged 3 to 14 years, the incidence of caries and mottled enamel varied greatly in the communities exposed to similar amounts of flourine in the drinking water. The incidence of caries varied inversely as the dental fluorosis index of the community. The optimal (sub-mottling) concentration of fluorine beneficial to dental health appeared to be 0.5 to 0.8 p.p.m. A method [99] for the removal of fluorine from water was also reported based on the property of rapid adsorption of fluorine by magnesium oxide added to the water while boiling.

K. Venkataramanan and V. Subrahmanyan and their associates submitted a series of reports on the treatment of brackish and fluoride-bearing waters to the Government of Madras under whose auspices a scheme of research on the subject was carried out at Bangalore during 1943-52.

Saline manipulation of brewing waters: The question of water supply of a brewery, as in the cases of most other industries, should be a first consideration when deciding on a new site. On behalf of a firm of brewers in South India, Iyer *et al.*[100] carried out some studies on the artificial treatment of certain well waters for manufacturing different classes of malt liquors, *e.g.*, pale ale, mild ale, mild beer, pale beer and bitters. The treated waters were found to yield beers of the desired types.

Treatment of boiler feed waters: Pillai [101] made some observations on the composition of boiler feed waters in a certain factory in South India, on the nature of the scale formed in the boilers, and on the possible method of controlling the formation of scale. The ion exchange material prepared from coffee husk (Ramakrishnan *et al.*, 1953, *loc. cit.*[96]) was found considerably efficient in removing calcium from 0.1% calcium chloride solution. It may perhaps be mentioned in this connection that in recent years a considerable amount of attention has been devoted

to studies[102-110] on the preparation and behaviour of ion exachange materials, which might find application in the treatment of water.

Basu [111] pointed out that in order to prevent the formation of scale in boilers the solubility of the scale-forming materials might be increased or they be kept in a colloidal condition by the addition of organic colloids; any remaining scale-forming substances might be absorbed on the active surfaces of a finely dispersed precipitate by adding to the water an aqueous solution of alkali metal salts of fatty acids or the aqueous extract of vegetable matter containing tannin. Puri [112] reviewed the various aspects of the treatment of boiler feed waters.

Treatment of waters for the silk industry: In certain filatures in South India considerable difficulty was experienced during the war years in regard to the production of high-grade raw silk. Commensurate with the quality of cocoons and the equipment employed in those filatures, the silk reeled was of a poor quality showing variations in colour and very hard gum spots. The investigations carried out by Pillai and Subrahmanyan [113,114] showed that the defects of the silk were due to the character and composition of the water used in the filature processes, particularly due to the alkaline reaction of the water. The alkali salts (bicarbonates) in the water were found (a) to react with sericin or the silk gum during the processes of cooking the cocoons and reeling the filaments, (b) to cause excessive dissolution of the gum, and (c) to change the colour of the silk, *e.g.*, from light greenish yellow to deep greenish yellow. The excessive amounts of the gum liberated were unevenly distributed or spread on the fibres as also concentrated at certain points causing hard gum spots, showing up patches of different shades of colour and adversely affecting the valuable properties of the silk, particularly its lustre, feel, evenness, neatness, cleanliness and its winding property. A simple, cheap and efficient method of treating such waters was developed by adding calculated amounts of sulphuric acid to the waters so as to neutralise or reduce their alkalinity to that of waters (*e.g.*, certain river waters) which were found to be quite suitable for reeling silk.

In this connection it may be of interest to note the observations of Pillay and Kaimal [115] who reported that waters from the tube-

wells at Alleppey and from certain other wells in Travancore containing alkali carbonates when used for cooking rice changed the colour of the rice, the red-bran rices developed red colour and the white-bran varieties developed a slightly yellowish tint. It was shown that this colour effect was due to the interaction of some organic matter present in the rice with the alkali carbonates present in the water and that addition of adequate amounts of mineral acids or some organic acids (*e.g.*, citric acid and tartaric acid) to the water before using it for cooking rice prevented the development of colour on the rice.

Radioactivity of mineral waters: Mathai [116] studied the radioactivity of samples of rocks and mineral waters from South Travancore employing an emanation electroscope. The activity of spring and well waters was found lower than that of those from similar sources in other parts of the world.

Fish farming in ponds, lakes, rivers and other waterways: Khosla [117] dealt with the various aspects of conservation and control of water and waterways, and pointed out that the large reservoirs formed for storage of water would afford immense possibilities for fish culture. Hora and Nair [118] reported the effects of the effluent from a factory manufacturing quinine on the pollution of streams and on the fish life. Mookerjee *et al.*[119] described their experiments indicating the optimum range of temperature of water for the fingerlings of major-carps of India. Basu [120] studied the oxygen requirements of Indian carp fingerlings. In a study of the pollution of two tanks near Calcutta with reference to fish mortality, Mukherjee and Bhattacharya [121] concluded that young fish could not tolerate high alkalinity accompanied by high concentrations of organic matter, even when the content of dissolved oxygen was appreciably higher than normal.

Chacko and Ganapathi [122] gave an account of the hydrobiological conditions of the Adyar river flowing south of Madras. The river contaminated with sewage and waste waters from a soap and glue factory in its upper reaches was found to recover from the polluting effects in the lower reaches where a fish farm was established. Ganapathi and Chacko and their co-workers [123-128] collected a considerable amount of evidence particularly on the

hydrobiological conditions and oxygen transactions in relation to fish culture in a number of waters in the Madras State. Bhaskaran [129] reviewed the modern trends in stream sanitation and discussed the relative values of the various chemical and biological indicators as parameters of pollution, including their bearing on public health, fisheries and development of other industries.

Pillai and Varier [130] artificially manured fish ponds with sodium nitrate and superphosphate and noted an increase in the plankton content and in the food value of the fish, especially in fat, protein and mineral matter. Vaas [131] presented analytical figures for nitrogen, oxygen, carbon dioxide and phosphorus of waters from fish ponds in Indonesia and discussed the use of manure, including sewage, for the fertilisation of the ponds. Krishna [132] observed that trout eggs and fry could develop normally in tap water with a fairly wide range of pH values (about 4.5 to 8.9) outside of which there was an abrupt rise in mortality. He also discussed the physiological effects of acidic and alkaline environments.

Hora [133] reported that the occurrence in recent years of *Hilsa toli*, a marine fish, in surface water of the Hooghly river would indicate that the water was becoming brackish. He discussed this aspect with reference to other reports of increase in salinity in the channels of the river below the Port of Calcutta.

SEWAGE

Development of *Vorticella* during aeration of sewage and its influence on the processes of clarification and sludge formation : While putting a new Activated Sludge Plant into operation in the premises of the Gun and Shell Factory at Cossipore, Calcutta, in September 1938, Pillai observed that the development in large numbers of the species of *Vorticella* was by far the most important influencing factor in the clarification of sewage and formation of active sludge (S. C. Pillai, Report, dated 30 September 1938 on the new Plant to the late Dr. G. J. Fowler who was then the Technical Representative of Messrs. Activated Sludge Ltd., London, for India and the East). Following this observation, the experiments carried out at Bangalore with thirteen prominent strains of bacteria

and four forms of protozoa (species of *Vorticella*, *Paramecium*, *Stylonychia* and *Colpoda*) from activated sludge showed that *Vorticella* was the most efficient organism in producing the cleanest effluent and the highest percentage of sludge.[134] A brief account of the further work done on the role of protozoa in sewage purification is given below.

On the occurrence and role of simple and colonial *Vorticellids*, *e.g.* **species of** *Epistylis*, *Opercularia*, *Carchesium* **and** *Zoothamnium*, **in activated sludge** : Continued observations over a period of years on the activated sludges in India showed that a number of species of protozoa belonging to these genera flourished in large numbers in the sludges throughout the year and that the flocculating action of activated sludge and its other properties were found to be largely due to the activity of the *Vorticellids*. In the marine activated sludge plant at Tuticorin where sea water has been used for flushing purposes (the treatment plant at this centre being the only one of its kind in the world) species of *Vorticella*, *Zoothamnium* and *Epistylis* which could tolerate the salts in sea water were found to develop and influence the purification process.[135-142]

Fowler [143] stated that nitrates in activated sludge acted catalytically by alternate oxidation and reduction, and that the activated sludge process could best be controlled by examination for a healthy growth of *Vorticella*.

Relative efficiency of bacteria and protozoa in the flocculation and oxidation of organic matter suspended in water : The flocculating activity of eighty-one different forms of bacteria isolated from water samples, soils, compost heaps, raw sewage and sludges and faeces of animals, such as cow, horse, rat, rabbit, dog and monkey, was compared individually and collectively with that of *Vorticella* sp. and *Epistylis* sp., employing soil suspension, sewage, compost extract, and mixtures of these materials as media. The bacteria, singly or together, showed relatively poor flocculating activity.[144] Nitrifying bacteria were present in all the sources examined, but nitrification was at a maximum only in presence of the protozoa.[145]

Role of protozoa in the concentration of nitrogen in the sludge during aerobic purification of sewage : Experiments with species of *Vorticella* and *Epistylis* showed that they concentrated nitrogen

in the sludge.[146] Raw sewage was found to contain almost all the essential amino acids and during purification by the activated sludge process the amino acids were concentrated in the sludge and the purified effluent was practically free from amino acids, as determined by the circular paper chromatographic technique.[147]

Relation of protozoa to 'bulking' of activated sludge: Inadequate air supply to the purification system led to the inactivity, disruption and decomposition of the protozoan cells and to the increase in bacterial numbers. The sludge was then found to get filled with gases due to bacterial action, to become light and 'rise' and gradually get dispersed.[148]

Influence of 'blood worms' (larvae of *Chironomus* sp.) on the protozoa and the purification process: These larvae, when they developed in large numbers in the activated sludge tank, were found to use the sludge flocs, including the useful protozoa, as material for constructing their characteristic tubes. The protozoa as also the purification process were thus adversely affected. Methods of control of the blood worms were also studied.[149]

Influence of fermenting yeasts on protozoal activity and purification of sewage: Evidence was adduced that the activity of the protozoa and the purification of sewage were adversely affected in presence of actively fermenting yeasts, and that this effect was due to the products of yeast fermentation rather than to the yeast cells themselves. In the light of these observations the cause of a somewhat mysterious break down in one of the earlier activated sludge plants in England where flushes of brewery waste were liable to enter the sewer was explained.[150]

Effect of different gases on protozoal activity and purification of sewage: When, instead of air or oxygen, gases such as carbon dioxide, nitrogen, hydrogen sulphide and the laboratory gas (mixture of kerosene gas and producer gas) were bubbled through sewage treated with the protozoa, the organisms were suppressed and there was no purification.[151] Some observations on the respiration, cultivation and nutrition of *Vorticella* sp. were also made.[152,153]

Nature of activated sludge and its bearing on dewatering and drying the material: Further evidence on the influence of the growth and

activity of *Vorticellids* on the physical structure and chemical composition of the sludge flocs was given. The main difficulty in dewatering and drying the sludge was found to be due to the presence of protozoa and other forms of life.[154] The use of filter mat (patented by Fowler) for dewatering the sludge was considered.[155]

Heukelekian and Gurbaxani [156] reported that when the protozoa in activated sludge were inactivated by certain physical and chemical agents, the purifying capacity of the sludge was decreased, the degree of clarification of sewage being more affected than the reduction in B.O.D. (Biochemical Oxygen Demand); that when pure culture zoogleal sludge was used, again clarification was not effective. They concluded that while protozoa assisted in clarification and oxidation of sewage, they were secondary in importance to bacteria.

Influence of activated sludge on pathogenic bacteria: *B typhosus*, *B. dysenteriae* and *Vibrio cholera* were found to be eliminated during intensive aeration of sewage as in the activated sludge process.[157]

Effect of antibiotics on activated sludge: The varying extent to which Aureomycin, Streptomycin and Penicillin adversely affected the functional activity of the sludge, *Epistylis* sp. and cysts of *Vorticella* sp. was indicated.[158] The observations would be useful in the understanding of the "very sensitive" and "extremely fickle" nature of the Activated Sludge Process known since the early days of the development of the Process.

Development of protozoa in land-filtered sewage effluent: Continued observations over a period of years showed that a large number particularly of the species of *Epistylis* and *Carchesium* developed and brought about oxidative changes in the effluent from the sewage farm at Madura.[159]

Protozoa as a link in the chain of life processes in the disposal of sewage and other organic wastes: An account of the more important micro-organisms and higher forms of life found during biological oxidation of organic matter in soil, sewage and other biological media was given and the significance of protozoa in the natural chain of living forms was indicated.[160,161] In this connection Fowler [162] published his observations which he had made while in Germany in 1913 on the life cycles in the polluted river of

Pegnitz into which Nuremberg sewage was discharged, and he pointed out that "*Vorticella* shows purified sewage." He[163] also published in 1947 a connected account of the developments which had taken place during the preceding decade in the field of sewage purification, emphasising the importance of the protozoa in the purification process and considering them as some newly observed links in the nitrogen cycle.

Role of protozoa in the purification of sewage by 'dilution': Experimental evidence was reported on the part played by ciliate protozoa such as *Vorticella* and *Paramecium* in the dilution method of sewage disposal.[164]

Influence of Vorticellids on the purification process in biological filters: Experiments were conducted with freshly broken pieces (about $\frac{1}{2}$" size) of granite, pumice, charcoal, coal as also glass beads (about $\frac{1}{8}$" dia.), coarse sand, clay, sand-clay mixtures, and sand sieved through different meshes (4-10 to 150-200). The filters (*e.g.*, "40-60" and "60-80" mesh filters in the earlier stages of filtration) providing maximum surface area and at the same time sufficient air space were found to yield much clearer effluents, and these filters contained a larger number of protozoa, such as the species of *Vorticella* and *Epistylis*.[165]

Occurrence of cysts of *Vorticella* as the only form of non-bacterial life in a certain decomposing sewage sludge: A sample of Calcutta sewage sludge examined in October 1951 was found to contain cysts of *Vorticella* sp. constituting practically the only type of non-bacterial life in the sludge. When this sludge (0.5 c.c.) was inoculated into heat-sterilised sewage (200 c.c.) and aerated, the cysts developed and the protozoan multiplied in large numbers. After aeration for 44 hours, each c. c. of the suspension contained 2,400 active *Vorticella* sp., and the sewage was found clarified.[166]

Development of Vorticellids and natural purification of flowing sewage: The raw sewage (10-14 m.g.d.) from the City of Bangalore allowed to flow down over a gradient land in the suburbs of the City, as it covered a distance of 5-6 miles, was found clarified and purified. At the point of purification abundant growth of species of *Epistylis* and *Carchesium* was invariably seen over a period of 21 months. The related experiments showed that these

protozoa were mainly responsible for the purification of the flowing sewage. [167]

Methods of analysis of sewage and sludges: Chakravarti and Som [168] made a comparative study of B.O.D. values of sewage with those obtained from calculation from the chemical oxygen demand. The method used for determining oxygen consumption was based on the dichromate method of Ingols and Murray (*Water and Sewage Wks.*, 1948, **95**,113). The value for oxygen consumption obtained by this method was found approximately equivalent to the value for 20-day B.O.D.; by multiplying this figure by 0.68 the approximate 5-day B.O.D. was obtained. The B.O.D. values obtained by calculation were found higher than those obtained by the standard B.O.D. test, presumably because of the chemical oxidation of substances such as cellulose and humus which were not oxidised biologically. When sewage was digested at different temperatures it was found that the calculated B. O. D. values increased with increase in temperature. Joshi [169] suggested methods of analysis of sewage sludges and composts.

Anaerobic treatment of sewage and other wastes and production of gas: In his account of the operation of the sewage-treatment plant at Dadar, Bombay, Modak [170] dealt with a number of points of practical interest, and reference may be made to the production of sludge gas containing 65% methane and to the suggestion that the gas might be utilised for steam-pressing the sludge which could then be safely used for fertilising crops. Bose [171] pointed out that sludge (about 210,000 gals.) pumped daily from the sedimentation tanks to the lagoons at the Calcutta sewage works might be digested and the sludge gas be used as fuel and the dried sludge as a fertiliser. Majumdar [172] also recommended digestion of the sludge as obtained from the settling tanks, as it could not be disposed of on land without causing nuisance.

Desai and Biswas [173] described two laboratory plants suitable for anaerobic fermentation of cow-dung, straw and waste material from sugarcane. One pound of dry material was found to yield 1.5 to 5 cu. ft. of fuel gas consisting of 45% methane, 45% carbon dioxide and 10% hydrogen. Successful operation without external heating required a high air temperature. The residue formed a

manure not inferior to ordinary stored farmyard manure. Desai[174] also reported that by resorting to anaerobic fermentation, the cattle dung could be used to serve both the purposes of producing fuel as well as manure. According to him, in order to obtain 60 to 80 c.ft. of gas, the average minimum daily requirement of a family of 4 to 5 persons, 1,500-2,000 lbs. of fresh dung should be fermented; the capacity of the fermenter should be 300-400 gallons and that of the gas holder should be about 60 c.ft. The original dung powder and the fermented residue were mixed with soil and the rate of nitrification determined; there was no difference. A mixed culture of bacteria capable of attacking pure cellulose under anaerobic conditions was also obtained. Acharya[175] gave an illustrated account of cow-dung gas plants.

Pasricha and Ghosh[176] described a simple method of obtaining anaerobic conditions using moistened iron filings.

Septic tanks: Ganguli[177] gave an account of septic tanks. Subrahmanyan[178] made observations on the design of small sewage disposal plants. Chopra[179] recommended a type of septic tank for railway hospitals and sewage disposal in dry places. Suryaprakasam[180] discussed the design and operation of Imhoff tanks for treatment of sewage with special reference to conditions in India. In an account of the methods of sewage disposal in India, Subrahmanyan[181] referred to the single-storey septic tanks used commonly even in communities of 40,000-50,000 people, and stated that such septic tanks except in Hyderabad were generally found overloaded. He also referred to the modern circular primary sedimentation tanks built at Calcutta and Bombay.

On the fertilising elements in sewage, sludges and other organic wastes: Pillai[182] indicated a method of growing plants (*e.g.*, rice, ragi, tomatoes, chillies, roses, tuberoses and marygold) by a system of what may be termed "hanging gardens" in the aeration tanks of the activated sludge installation. It was pointed out that by this technique some of the fundamental problems of plant nutrition, more especially the influence of micro-organisms, could be studied since the changes which proceed in activated sludge are similar to those in fertile soil.

Fowler[183] dealt with the agricultural utilisation of sludge

from several types of sewage treatment plants and production of sludge which would not create nuisance. He [184] indicated the possibilities of increasing the production of potassium nitrate in India from ammoniacal waste waters and from old trenching grounds, and he [185] discusssd the utilisation of sewage effluent for the recovery of sodium, potassium and phosphorus compounds and of sludge for the preparation of compost.

Joshi [186] reported that a large proportion of the phosphorus in sewage was in the inorganic form, soluble in water and was therefore in an available form. He [187] found that sewage sludges were somewhat richer than sewage in organic phosphorus but had a lower content of water-soluble phosphorus compounds. In a further study of the role of water-soluble phosphate as an aspect of sewage irrigation, Joshi *et al.*[188] observed that irrigation of a ragi crop with water containing added nitrogen resulted in a significant increase in the nitrogen content of both grain and straw, but no increase in the content of phosphorus; and that irrigation with water to which only the soluble phosphate was added, however, increased both the nitrogen and phosphorus contents. This effect differs from that of applying fertilisers containing phosphates to soil before the crops are grown, which has been found to cause a decrease in the content of nitrogen. Pillai *et al.*[189] drew attention to the very considerable loss of nitrogen from sewage and to the possible methods of its conservation and utilisation for the production of crops.

In continuation of the earlier studies by C. N. Acharya and V. Subrahmanyan on composting and conversion of town refuse into manure (Reports to the Indian Council of Agricultural Research under whose auspices the work was done at Bangalore), an extensive programme for the manufacture of compost manure was carried out in the country as a nation-wide effort. [190] Further observations on the biochemical [191,192] and hygienic aspects of composting, *e.g.*, on the control of fly breeding, [193,194] were also made.

Utilisation of sullage, sewage and sludges for crop production: Early in 1940, S. C. Pillai submitted to the late Dr. G. J. Fowler an account of the land treatment of sewage, the behaviour of the soil

and crops to sewage irrigation and related aspects of the sewage farm at Baroda which is one of the oldest sewage farms in the country.

Rao [195,196] observed that irrigation of certain rice fields at Orissa with sullage led to a considerable decrease in the species of *Anopheles* mosquitoes, particularly the carriers of malaria. Talait [197] described the practice of diluting sewage with water for crop irrigation at Poona.

During 1942-50, a systematic investigation into land treatment of sewage and crop production was carried out at Bangalore under the auspices of the Indian Council of Agricultural Research. The various aspects of sewage farming studied include : the soils and crops suited to sewage irrigation ; the fertilising value of diluted sewage and of mixtures of sewage and waste waters from the textile industry, with special reference to the conditions obtained at Ahmedabad ; the effect of application of sewage during different stages of plant growth ; the residual effect of sewage on subsequent crops ; the decomposition of sewage in soil ; the accumulation of unavailable phosphorus in sewaged soils ; the viability of pathogenic organisms during sewage treatment ; the nature and extent of infection carried by sewage-grown crops ; the cause and control of ' sewage-sickness ' of soil ; the use of aerated sludge as a fertiliser and for feeding animals ; the effect of sea water on the activated sludge process ; drainage from sewage-irrigated soils and the quality of effluents, with special reference to the conditions at Madura ; and the culture of fish in the effluents from sewage farms.[198-202] Results of further studies by others on activated sludge as a fertiliser,[203] on the growth of sugarcane on land irrigated with sewage,[204] and on other aspects of sewage farming [205] were also made available.

Utilisation of sewage and sewage effluents for fish culture : Fowler[206] made recommendations concerning the required quality of the effluent to be used for fish culture. Nair,[207,208] and Bose [209] studied the effect of discharge of Calcutta sewage into the Kulti river on the fish life in the river and also discussed other aspects of utilisation of sewage for fish culture. Basu [210] gave an account of the ecology of sewage-irrigated fisheries in the Bidyadhari spill area, with special reference to the bionomics of carps cultured therein.

Ganapathi and Chacko [211] estimated that sewage from a population of 1000 would require a fish pond one acre in area and 5 ft. in depth ; that after about 40 hours the oxygen demand would be reduced by 40-50% ; and that an annual crop of 3,000 lbs. per acre could be expected. Bhaskaran [212] discussed the oxygen balance in fish ponds fertilised with organic manures, particularly sewage.

Rural sanitation : Ghosh [213] chalked out a programme for rural sanitation, which included protection and repair of existing tube-wells, chlorination of open wells and provision of adequate cover for such wells, provision of water storage tanks for drinking purposes, installation of village drains, installation of soak-pits for every 4 houses, construction of bore-hole latrines on selected sites, use of sullage as an irrigant for kitchen gardens and use of cow-shed wastes as compost manure. He [214] further reported that septic tanks would be satisfactory for communities with populations not exceeding 150. He deprecated the practice prevalent in Bengal of installing septic tanks indiscriminately and pointed out that before installing a septic tank it was important to ensure that the soil to which the effluent would be discharged was sufficiently absorbent and that the water table was sufficiently low in the subsoil to prevent water-logging or rising of the effluent to the surface. In considering the need for the construction of family latrines [215] the design and cost of three types of latrines, *viz.*, bore-hole, pit and septic tank, that could be used under different conditions were given.

Subrahmanyan [216] discussed the design of sewage-treatment plants for small communities, with special reference to the conditions in the semi-rural districts of India.

INDUSTRIAL WASTES

Treatment and utilisation of waste materials from the sugar industry : In continuation of the earlier studies by T. R. Bhaskaran, S. C. Pillai and V. Subrahmanyan on the treatment and utilisation of cane molasses, with particular reference to agricultural utilisation (*J. Indian Inst. Sci.*, 1938, **21A**, 27 ; *Ibid.*, 1940, **22A**, 127, 139, 149, and 237), further work was reported on the nature and extent of loss of the biologically fixed nitrogen following the application

of molasses-lime product to soil and on the role of straw in the conservation of the fixed nitrogen. [217,218]

At the symposium on the utilisation of industrial wastes held by the National Chemical Laboratory, Poona, in February, 1952 the recovery of potassium sulphate from cane mud was discussed.[219] Daruvalla and Kamath [220] developed a process for the production of mineral wool from a mixture of filter press mud and sand melted in a furnace at 1,000 to 1,600°C.

Iyer *et al.*[221] studied the character and composition of the effluents from sugar factory, vegetable ghee factory and wood ground mill located at the same site in a town in North India. In regard to the purification of these trade effluents along with sewage by the activated sludge process, it was observed that the purification was dependent on the reaction of the mixture of these effluents and domestic sewage, which should be nearly neutral.

Treatment and utilisation of waste waters from the textile industry : An extensive study of the various aspects of the treatment and agricultural utilisation of the liquid wastes from the cotton textile mills at Ahmedabad was made by V. Subrahmanyan and his associates who submitted a number of reports on the subject during 1942-49 to the municipality of Ahmedabad, to the Council of Scientific and Industrial Research and to the Indian Council of Agricultural Research, who sponsored the scheme of research.

Iyer *et al.*[222] observed that ordinarily the waste waters from textile mills should be diluted over six times with domestic sewage and those from breweries be diluted over thirty times with domestic sewage in order that purification might proceed in the sewage-trade effluent mixtures.

Effect of tannery waste waters on soil conditions and plant growth : In an investigation into the loss of productive capacity of about 100 acres of paddy fields in the neighbourhood of a tannery near Madras, Iyer *et al.*[223,224] analysed a number of samples of tannery effluents, soils of the paddy fields, the subsoil waters and related materials, and collected evidence that the salts and other constituents of tannery effluents were responsible for the deterioration of the soil in the affected belt of the land and for the failure of crops. In this connection reference may be made to the observations of

Asghar and Dhavan [225] on the effect of the ratio of sodium to calcium salts on the quality of irrigation water.

Treatment and disposal of the effluents from paper mills: Iyer *et al.*[226] studied the character and composition of the effluent from digesters in a certain paper mill in North India. A suitable method of pre-treatment of the effluent was recommended before the material could be diluted with domestic sewage and purified by aerobic methods, *e.g.*, the activated sludge process. Ganapathi and Chacko [227] recorded that combined effluent from bamboo, rag and waste paper pulping operations created heavy local pollution of the Godavari river at Rajahmundry; as a control measure they suggested rough sand filtration or impounding with discharge during flood stage.

Treatment and utilisation of lac wastes: Seth and Bhaskaran [228] reported that the lac waste waters could be treated by coagulation to remove gelatinous matter, followed by biological filtration. The filter effluent was of good quality and the sludge from the filters could be used to produce gas, or be used as a fertiliser, or for recovery of lac or lac wax.

Treatment and disposal of the effluent from producer gas plants: Thampy [229] tried two methods of removal of phenolic bodies from the waste, using benzene and butyl acetate. The use of benzene for phenol recovery would be economical if the cost of steam was low.

Recovery of nickel and zinc from silver refinery waste liquors: Banerjee and Banerjee [230] reported the results of their studies on the recovery of nickel by electrodeposition. Dutta and Banerjee [231] described chemical methods of recovery of nickel.

Treatment and utilisation of wastes from the film industry: The observations of Seth [232] would suggest that from the solid wastes from the film industry both cellulosic material and metallic silver could be recovered and that from the liquid wastes metallic silver could be recovered.

Treatment and utilisation of the pulpy residue from the coffee bean and the husk: The possibility of utilising the pulpy residue from the coffee bean as cattle feed was investigated to some extent at the Indian Veterinary Research Institute, Izatnagar.[233]

Natarajan *et al.*[234] suggested the utilisation of coffee husk in

the preparation of coffee powder, for blending in place of chicory. Ramakrishnan *et al.* (*loc. cit.*[96]) studied the characteristics and composition of five types of coffee husk and described a method of treating and utilising the material for preparing an ion exchange carbon which could be used for treating hard and fluoride-bearing waters.

Some aspects of industrial hygiene: Subrahmanyan and Majumder reported the results of their studies on the environmental conditions within jute mills [235] and in printing presses. [236] Rao [237] conducted a study of the comfort range in tropical Calcutta. Sabnis [238] collected evidence on lead poisoning in a pigment manufacturing concern.

Investigations were carried out by the organisation of the Chief Adviser of Factories, New Delhi, on the health hazards in the dichromate manufacturing plants in India and in the chromite mining and concentrating industries,[239] on the working conditions in the storage battery manufacturing industry, [240] and on silicosis in mica mining in Bihar.[241]

Finally, reference may be made to the tragic death of Mr. K. Subrahmanyan, Professor of Sanitary Engineering in the All-India Institute of Hygiene and Public Health, Calcutta, who was killed in an air crash at Dum Dum on 21 November, 1951 and to the passing away of Dr. Gilbert J Fowler on 21 March, 1953. Apart from his assistance in drafting the chapters on Environmental Hygiene in the Bhore Committee's Report and in drawing up the final Report of the Environmental Hygiene Committee set up by the Government of India, Professor Subrahmanyan rendered valuable services to the World Health Organisation in which as an international expert he helped in tackling the numerous problems bearing on environmental hygiene confronting the South-East Asia Region. Dr. Fowler was an outstanding figure in the field of sanitation biochemistry. He will be principally remembered in this country as a pioneer who started biochemical research, more especially on the treatment and utilisation of waste materials. His association with India began in 1906 as a consultant in matters bearing on disposal of sewage and trade effluents, and from 1916 when he became the Professor of Biochemistry in the Indian

Institute of Science and settled down in the country, to the last days of his life, he was closely connected with the scientific studies and practical developments in sanitation. A number of articles[242-248] were published dealing with the life and work of Professor Subrahmanyan and of Dr. Fowler.

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65	,, 70	Indian Med. Sci.,	Indian J. Med. Sci..
,,	,, 76	year of publication 1953	
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,,	,, 33	page No. for publication, 42	
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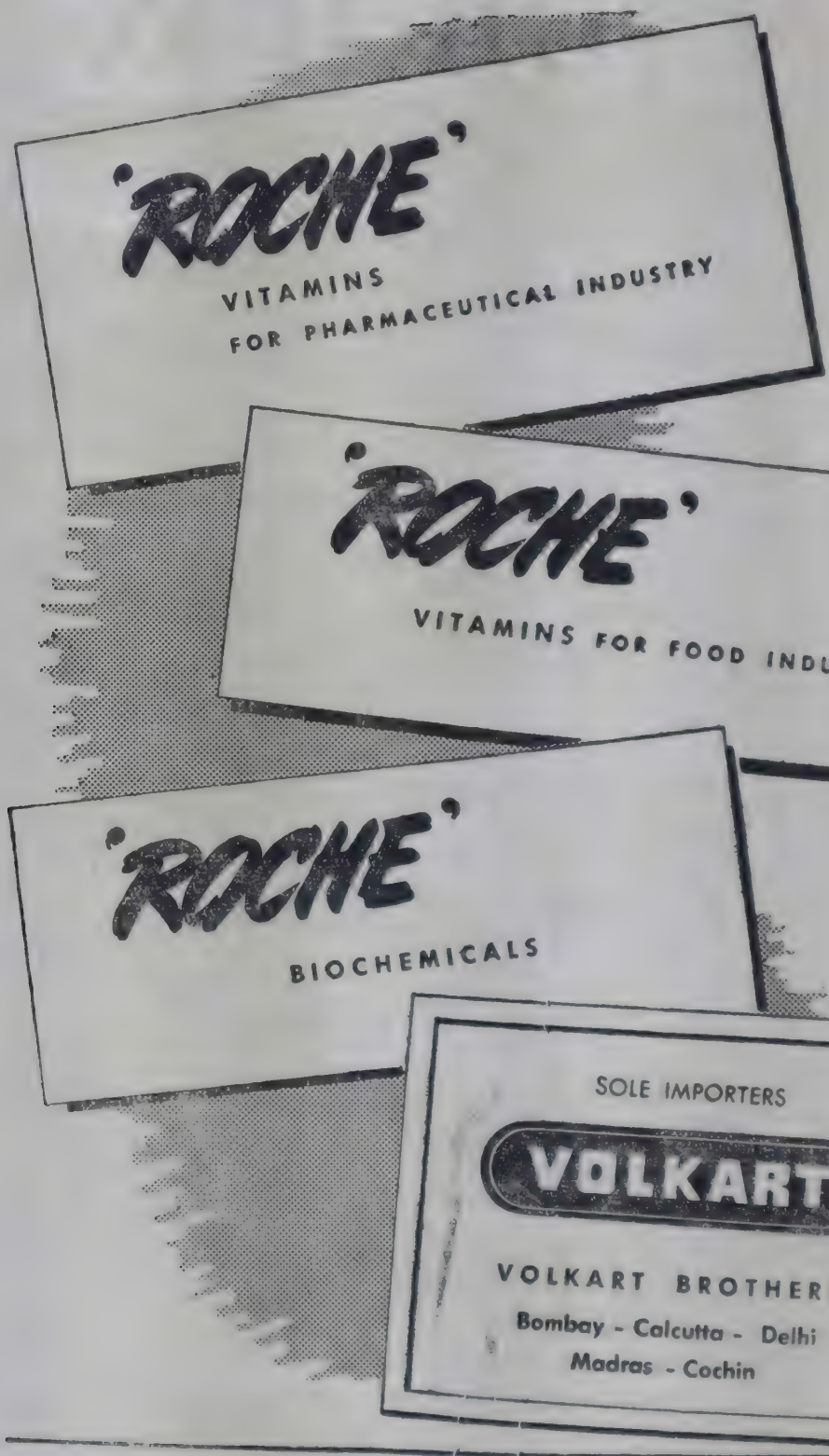
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